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(57) Abstract

The present invention relates to 1,4–benzodiazepines or 1,4–benzothiazepines derivatized with a peptide that can inhibit the interaction between annexin and annexin binding proteins. In particular, the present invention relates to 1,4–benzodiazepines or 1,4–benzothiazepines derivatives that can inhibit the interaction between annexin and viral proteins that bind annexins such as the HBsAg protein of HBV, glycoprotein B of the cytomegalovirus or any annexin binding protein from the influenza virus. These 1,4–benzodiazepines or 1,4–benzothiazepines derivatives can be used to prevent or treat diseases in which interactions between annexin family members and annexin binding proteins are involved such as HBV and/or HDV infections, cytomegalovirus infections or influenza virus infections.

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BENZODIAZEPINES AND BENZOTHIAZEPINES DERIVATIVES AND HBSAG PEPTIDES BINDING TO ANNEXINS, THEIR COMPOSITIONS AND USE

FIELD OF THE INVENTION

The present invention relates to the finding that 1,4-benzodiazepines and 1,4-benzothiazepines derivatized with a peptide chain inhibit binding of HBsAg to annexin V. Thus the present invention concerns such molecules and their use to treat infections with hepatitis B virus and hepatitis delta virus, or any other disease in which a protein interaction with annexin family members is involved.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) belongs to the Hepadnaviridae, which are characterised by a significant hepatotropism and species specificity. Hepatitis delta virus (HDV) represents a naturally occurring subviral satellite of HBV (Rizetto et al, 1986). HBV causes major medical problems, such as chronic liver disease and hepatocellular carcinoma (Schroder and Zentgraf, 1990). HDV superinfection is usually more severe compared to HBV infection solely. It is estimated that there are 300 million human carriers of the virus worldwide, while in the US only 70,000 carriers are coinfected with HDV.

Within the HBV genome, and more particularly within the S gene (see also next paragraph), there exists a natural sequence variation. Genotypes A to F of HBV are designated based on this sequence divergence (for review, see Magnius and Norder, 1995).

The HBV envelope consists of three related glycoproteins, termed hepatitis B surface antigens (HBsAg), which are the product of the S gene: 1) the "small" transmembrane protein, also termed major protein or small S-protein, composed of 226 amino acids (aa), 2) the "middle" protein which comprises the small S-protein and 55 additional amino acids at the N-terminus corresponding to the pre-S2 region of the S gene, and 3) the "large" protein composed of 389 or 400 amino acids corresponding to the following regions: S + pre-S2 + pre-S1 (108-119 N-

terminal aa) (Heerman et al., 1984; Robinson et al., 1987). The envelope of HDV is also entirely derived from HBV and consists predominantly of small HBsAg, 5-10% of middle HBsAg and no or less then 1% of large HBsAg (Bonino et al., 1986).

Influenza viruses cause recurrent epidemics and even global pandemics. During these outbreaks absenteeism from work leads to high economic losses and is accompanied with increases of hospitalisation and even death due to respiratory diseases (for review Cox and Fukuda, 1998).

Cytomegalovirus is a member of the herpes virus family and is an important pathogen in immunocompromised individuals such as AIDS patients. Within the envelope of herpes viruses a protein referred to as glycoprotein B is present. This protein has been shown to be essential for infectivity including host cell entry and cell-to-cell spread (Cai et al., 1988).

Annexin V (also termed endonexin II, placental anticoagulant protein, PP4 or lipocortin V) is a member of the family of structurally related Ca^{2+}-dependent phospholipid-binding proteins, known as annexins, which have molecular weights between 32 and 67 kDa (Klee, 1988; Zaks and Creutz, 1990). Annexin V is found in various tissues such as liver, spleen, lung, intestine and placenta (Walker et al., 1990). The protein has been described to bind, in a Ca^{2+}-dependent manner, to placental membranes (Haigler et al., 1987) and to inhibit blood coagulation (Grundmann et al., 1988) and phospholipase A2 activity *in vitro* (Pepinsky et al., 1988). Other investigators have demonstrated that annexin V behaves like an integral membrane protein and forms calcium-selective cation channels (Rojas et al., 1990; Bianchi et al., 1992).

Recently it has been shown that 1,4-benzodiazepines (Hofmann et al., 1998) and 1,4-benzothiazepines (Kaneko et al., 1997a, b) can bind to annexin V. Annexin II is related to annexin V and also binds 1,4-benzodiazepines (Hofmann et al., 1998). Annexins also display a large variety of binding to non-viral proteins such as collagen, tenascin, actin, synapsin, calspectin, insulin receptor, plasminogen,... (for review see Sheldon and Chen, 1996).

We have recently shown that annexin V, present on human liver plasma membranes, specifically binds to "small" HBsAg in a Ca^{2+}-dependent manner (Hertogs et al., 1993; WO 94/01554). The receptor-ligand relationship between HBsAg and annexin V is further supported by the observation that rabbits, immunised with native human liver annexin V or recombinant annexin V, or chickens, immunised with $F(ab')_2$-fragments of rabbit anti-annexin V IgG, spontaneously develop anti-idiotypic antibodies (Ab2) which specifically recognise HBsAg

(Hertogs et al., 1994). We also demonstrated that HDV particles are binding to annexin V via the HBsAg containing envelope of HBV (de Bruin et al., 1994). The mapping of the sites on HBsAg which are involved in annexin V binding are described in WO 97/07268. These sites reside within the region composed of amino acids 100 to 160 of "small" HBsAg, which has been predicted to be located on the outer surface of the virus (for review, see Berting et al., 1995).

The binding of influenza virus to annexin V (Huang et al., 1996) and of cytomegalovirus to annexin II (Pietropaolo and Compton, 1997) has also been demonstrated.

It is clear from the literature cited above that HBV and/or HDV infection as well as other viral infections such as influenza and cytomegalovirus infections, are still a major health problem. The development of new drugs interfering with the life cycle of HBV and/or HDV or of these other viruses, is therefore still a major need. Compounds that bind annexin in such a way that the binding of HBV and/or HDV or of these other viruses to annexin is inhibited, could be good candidates for interfering with the life cycle of these viruses and consequently, for the use as a drug to treat viral infections. Therefore, there is an urgent need to provide molecules that specifically interact with annexin, simultaneously inhibiting the interaction of HBV and/or HDV or of these other viruses with annexin.

AIMS OF THE INVENTION

It is an aim of the present invention to inhibit the interaction of annexin with an annexin binding protein.

It is a more specific aim of the present invention to inhibit the interaction of annexin with a viral protein that is able to bind annexin.

It is another more specific aim of the present invention to inhibit the interaction of annexin V with the surface antigen of HBV.

It is another more specific aim of the present invention to inhibit the interaction of annexin II with the glycoprotein B of the cytomegalovirus.

It is another more specific aim of the present invention to inhibit the interaction of annexin V with a protein derived from any influenza strain that binds annexin V.

It is another aim of the present invention to prevent or treat any disease in which interactions

between annexin family members and annexin binding proteins are involved.

It is a more specific aim of the present invention to prevent or treat any viral disease in which interactions between annexin family members and viral proteins are involved.

It is another more specific aim of the present invention to prevent or treat HBV and/or HDV infections.

It is another more specific aim of the present invention to prevent or treat cytomegalovirus infections.

It is another more specific aim of the present invention to prevent or treat influenza virus infections.

All the aims of the present invention are considered to have been met by the embodiments as set out below.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein draws on previously published work and pending patent applications. By way of example, such work consists of scientific papers, patents or pending patent applications. All these publications and applications, cited previously or below are hereby incorporated by reference.

The present invention relates to 1,4-benzodiazepines and 1,4-benzothiazepines derivatized with one or more peptides.

In particular, the present invention relates to 1,4-benzodiazepines and 1,4-benzothiazepines derivatized with one or more peptides such that the derivative inhibits the interaction between annexin and certain annexin binding proteins.

1,4-Benzodiazepines and 1,4-benzothiazepines which do bind annexin V, a molecule involved in the viral entry of HBV, do not inhibit the binding of HBV to annexin V. The present invention is based on the surprising finding that upon derivation of these compounds with a peptide ligand, the interaction between annexin V and the HBsAg of HBV can be inhibited. These new derivatives of 1,4-benzodiazepines and 1,4-benzothiazepines are therefore a new lead in the discovery of drugs interfering with the life cycle of HBV and/or HDV and other viruses binding to annexins in general, or more specifically, to annexin V. In general these molecules may interfere

with any interaction of an annexin with another protein. By selective derivation of the 1,4-benzodiazepines or 1,4-benzothiazepines, any of these interactions may be targeted in a specific way.

The term "annexin" (also referred to as endonexin II, placental anticoagulant protein, PP4 or lipocortin V) refers to any member of the family of structurally related Ca^{2+}-dependent phospholipid-binding proteins, known as annexins, which have molecular weights between 32 and 67 kDa (Klee, 1988; Zaks and Creutz, 1990). More specifically, the annexin can be annexin V shown to bind to the HBsAg of HBV and to the influenza virus, or annexin II known to bind to glycoprotein B of cytomegalovirus. But the annexins referred to in the present invention are not restricted to these annexins, but can also be any other annexin that binds with an annexin binding protein.

The 1,4-benzodiazepine or 1,4-benzothiazepine may be any modification known in the art such as the ones mentioned in WO 94/11360 and WO 92/12148. Preferably, the 1,4-benzodiazepine or 1,4-benzothiazepine has a structure as shown in formula I (figure 1), wherein:

* at position 1: - benzothiazepines have a sulphur atom (X represents S); X may also be SO_n with n = 0, 1, 2;
  - benzodiazepines have a nitrogen atom (X represents N) which allows additional modifications with a peptide as defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, linked via the C-terminus of the peptide or linked via the N-terminus of the peptide after additional modification of position 1 with carboxymethyl (-CH_2COOH). In case N is not modified with a peptide, modification can be H, alkyl, phenyl, -COZ in which Z stands for H, alkyl, phenyl or substituted phenyl;

* positions 1, 2, 3, 4, 5 may form double bonds with adjacent positions; if this is the case the side chains R_2, R_4, R_5 or R_7 are non-existing;

* R_1 and/or R_2 may represent at position 2 and/or 2' H, amine, alkyl or position 2 may be oxidised (=O). In case R_1 and/or R_2 is amine, this may be further substituted with a peptide defined in the

claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, either directly linked via the C-terminus of the peptide or linked to the N-terminus of the peptide via a linker such as glutaraldehyde or succinicanhydride;

* R_3 and/or R_4 may represent at position 3 and/or 3' H, amine or alkyl. In case R_3 and/or R_4 is amine, this may be further substituted with a peptide defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, either directly linked via the C-terminus of the peptide or linked to the N-terminus of the peptide via a linker such as glutaraldehyde or succinicanhydride;

* R_5 may represent at position 4 a side chain defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein", or part thereof, either directly linked via the C-terminus of the peptide or linked via the N-terminus of the peptide after additional modification of position 4 with carboxymethyl ($-CH_2COOH$). In case R_5 is not a peptide as defined by the claims, R_5 may represent H, alkyl, $CO-R_{12}$ or 3-(1-(4-benzyl)piperidinyl)propionyl);

* R_6 and/or R_7 represent H or alkyl or phenyl, possibly further substituted with alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl;

* R_8, R_9, R_{10} and/or R_{11} represent H, alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl;

* R_{12} represents H or alkyl or phenyl possibly further substituted with alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl.

The 1,4-benzodiazepine or 1,4-benzothiazepine used for the preparation of a molecule of the present invention can also be any pharmaceutically acceptable salt of this formula. The 1,4 benzodiazepine used for the preparation of a molecule of the present invention may be any derivative known in the art such as but not limited to alprazolam, bromazepam, chloordiazepoxide, clobazam, clonazepam, clorazepate, diazepam, fludiazepam, flunitrazepam, flurazepam, lorazepam, nitrazepam, oxazepam, temazepam, triazolam, BDA 250, BDA 452, BDA

753 (see also figure 6 and Hoffman et al., 1998). However, the compound of the present invention is not BDA753, a compound according to formula II (FR2479818; figure 2) or a compound according to formula III (Nachman et al., 1998; figure 3). The 1,4 benzothiazepine may be any known derivative known in the art such as but not limited to K-201 (Kaneko et al., 1997a).

According to the present invention, these 1,4-benzodiazepine or 1,4-benzothiazepine are derivatized with a polypeptide that can be linked at positions 2, 2', 3 and/or 3' (in formula I) after modification of said positions with an amine (two peptides may be linked at the same position). The peptide can be linked to the amine, either directly via the C-terminus of the peptide, or the peptide can be linked via the N-terminus of the peptide by use of a linker.

The linker can be any linker that allows linking of two amine residues. Preferably the linker is a dialdehyde such as glutaraldehyde or an anhydride such as succinicanhydride. Other possible linkers can also supplied by Pierce (Rockford, IL, USA). Examples of the basic reaction for linking the peptide to the 1,4-benzodiazepine by use of a linker are shown in figures 9 and 10.

The polypeptide can also be linked to positions 1 and/or 4 (in formula I). In this case, the peptide is linked either via the C-terminus of the peptide or the peptide is linked via the N-terminus after additional modification of respectively position 1 and/or 4 with carboxymethyl (-CH_2COOH).

In a preferred embodiment, the peptide is linked to position 1 and/or 3 of 1,4-benzodiazepine substituted with a phenyl at position 5. One peptide, R, can be linked via its C-terminal to position 3 of said 1,4-benzodiazepine after modification of position 3 with amine, or one peptide, R', can be linked via its N-terminal to position 1 of said 1,4-benzodiazepine after modification of position 1 with carboxymethyl, or two peptides, R and R', are linked to respectively positions 3 and 1 of said 1,4-benzodiazepine. Accordingly, the present invention relates to said 1,4-benzodiazepine derivatized with one or two peptides, R and/or R', such that R, R' and/or R + R' represent a peptide containing an annexin binding epitope of an annexin binding protein or part thereof. The 1,4-benzodiazepine then has a structure as shown in formula IV (figure 4), wherein:

* R, R' and/or R + R' constitute a peptide containing an annexin binding epitope of an annexin binding protein or part thereof. In case only R is a peptide, position 1 is methylated or carboxymethylated. In case only R' is a peptide, position 3 contains amine;

* X represents the N-terminus of the peptide which may be modified with -$COCH_3$;

* Y represents the C-terminus of the peptide which may be modified with -NH_2 or -Lysine-Biotine.

A building block that can be used for the synthesis of said 1,4-benzodiazepine derivative by Fmoc based peptide synthesis can be obtained from Neosystem (Strasbourg, France).

In another embodiment of the invention, the peptide, R, is linked via its N-terminal to position 3 of said 1,4-benzodiazepine (substituted with phenyl at position 5), by use of a linker as described above. In this case, the nitrogen atom at position 1 (formula I) is methylated or carboxymethylated. Accordingly the present invention relates to a compound according to formula V derivatized with a peptide, R, containing an annexin binding epitope of an annexin binding protein or part thereof.

The polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine can be any polypeptide that comprises an annexin binding epitope derived from a protein known to bind annexin.

The term "annexin binding protein" refers to any protein that is able to form a complex with annexin.

The term "annexin binding epitope" refers to that domain of the annexin binding protein that specifically interacts with annexin to form the annexin binding protein - annexin complex. The "annexin binding epitope" comprises at least 2, preferably 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35 or more amino acids of the annexin binding protein. Epitopes can be determined by any of the techniques known in the art or may be predicted by a variety of computer prediction models known in the art.

The terms "polypeptide" or "peptide" are used interchangeable and refer to a polymer of amino acids which is equal or similar to a part of the protein where it is derived from. The polypeptide can consist of any number of amino acids, preferably the polypeptide consists of less than 13 amino acids, preferably of 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acids such that an annexin binding epitope is contained within the polypeptide. In case the compound of the invention has a structure according to formula IV, it is also possible that the peptide R or R'

consists of only 1 amino acids and/or that the annexin binding epitope is contained within the polypeptide that represents R+R'. It should be noted that the polypeptides which are derived from the annexin binding protein and which form a binding region to an annexin, might be part of a conformational binding region and should thus not be composed of a contiguous amino acid sequence of the annexin binding protein.

The polypeptide can be prepared by any method known in the art such as classical chemical synthesis, as described by Houbenweyl (1974) and Atherton and Shepard (1989), or by means of recombinant DNA techniques as described by Maniatis et al. (1982). The term "polypeptide" does not refer to, nor does it exclude, post-translational modifications of the polypeptide such as glycosylation, acetylation, phosphorylation, modifications with fatty acids and the like. Included within the definition are, for example, polypeptides containing one or more analogues of an amino acid (including unnatural amino acids or D-isoforms), polypeptides with substituted linkages, mutated versions or natural sequence variations of the polypeptides (for example, peptide variants of the HBsAg protein of HBV, corresponding to the genotypes A to F of HBV, as indicated above), polypeptides containing disulphide bounds between cysteine residues, reverso-inverso polypeptides (such as described by Guichard et al., 1994), as well as other modifications known in the art.

In a specific embodiment, the polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine is derived from a viral protein that is known to bind annexin. The term "polypeptide derived from a viral protein" refers to a polymer of amino acids which is equal or similar to a part of the viral protein where it is derived from and which contains an annexin binding epitope of the viral protein.

In another specific embodiment, the polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine is derived from the HBsAg protein of HBV, which is a known antigen (Heerman et al., 1984; Robinson et al., 1987). As used herein, the term "polypeptide derived from the HBsAg protein of HBV" refers to a polypeptide having an amino acid sequence which is equal or similar to a part of the amino acid sequence of "small" HBsAg and which contains an annexin binding epitope of HBsAg. It should be clear that the "polypeptides derived from the HBsAg protein of HBV" can be derived from any genotype of HBV (genotype A to F, Magnius and Norder 1995).

In another specific embodiment of the present invention, the polypeptide linked to the 1,4-

benzodiazepine or 1,4-benzothiazepine is derived from glycoprotein B of the cytomegalovirus. The term "polypeptide derived from glycoprotein B" refers to a polymer of amino acids which is equal or similar to a part of glycoprotein B of the cytomegalovirus and which contains an annexin binding epitope of glycoprotein B.

In another specific embodiment of the present invention, the polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine is derived from a protein from any influenza strain on the condition that this influenza protein binds annexin V. The term "polypeptide derived from a protein from an influenza strain" refers to a polymer of amino acids which is equal or similar to a part of the protein from the influenza strain and which contains an annexin binding epitope of the influenza protein.

Furthermore, the present invention aims at providing a 1,4-benzodiazepine or 1,4-benzothiazepine with a peptide derivation comprising any combination of peptides as defined above or derived of any mutated strain of HBV and/or HDV, cytomegalovirus or influenza virus.

In a specific embodiment, the present invention provides a 1,4-benzodiazepine or 1,4-benzothiazepine molecule derivatized with a peptide that comprises less than 13 amino acids containing an annexin binding epitope of one of the following regions of the HBV surface antigen:

KTCTTPAQGN (SEQ ID NO 1)
FAKYLWEWASVR (SEQ ID NO 2)

which have been described as being involved in the interaction of HBsAg with annexin V (WO 97/07268 and present invention).

More specifically the polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine derivative of the present invention relates to polypeptides comprising 2-10 amino acids derived from the sequence spanning amino acid positions 122 to 131 of the HBsAg of HBV, shown above as SEQ ID NO 1. More specifically, to peptides comprising or consisting of the following amino acid (aa) sequences: aa 122-123, aa 123-124, aa 124-125, aa 125-126, aa 126-127, aa 127-128, aa 128-129, aa 129-130, aa 130-131, aa 122-124, aa 123-125, aa 124-126, aa 125-127, aa 126-128, aa 127-129, aa 128-130, aa 129-131, aa 122-125, aa 123-126, aa 124-127, aa 125-128, aa 126-129, aa 127-130, aa 128-131, aa 122-126, aa 123-127, aa 124-128, aa 125-129, aa 126-130, aa 127-131, aa 122-127, aa 123-128, aa 124-129, aa 125-130, aa 126-131, aa 122-128, aa 123-

129, aa 124-130, aa 125-131, aa 122-129, aa 123-130, aa 124-131, aa 122- 130, aa 123-131, aa 122-131. Similarly, the polypeptide linked to the 1,4-benzodiazepine or 1,4-benzothiazepine derivative of the present invention specifically relates to the polypeptides comprising 2 to 12 amino acids derived from the sequence spanning amino acid positions 158 to 169 of the HBsAg of HBV, shown above as SEQ ID NO 2. More specifically, to peptides comprising or consisting of the following amino acid (aa) sequences: aa 158-159, aa 159-160, aa 160-161, aa 161-162, aa 162-163, aa 163-164, aa 164-165, aa 165-166, aa 166-167, aa 167-168, aa 168-169, aa 158-160, aa 159-161, aa 160-162, aa 161-163, aa 162-164, aa 163-165, aa 164-166, aa 165-167, aa 166-168, aa 167-169, aa 158-161, aa 159-162, aa 160-163, aa 161-164, aa 162-165, aa 163-166, aa 164-167, aa 165-168, aa 166-169, aa 158-162, aa 159-163, aa 160-164, aa 161-165, aa 162-166, aa 163-167, aa 164-168, aa 165-169, aa 158-163, aa 159-164, aa 160-165, aa 161-166, aa 162-167, aa 163-168, aa 164-169, aa 158-164, aa 159-165, aa 160-166, aa 161-167, aa 162-168, aa 163-169, aa 158-165, aa 159-166, aa 160-167, aa 161-168, aa 162-169, aa 158-166, aa 159-167, aa 160-168, aa 161-169, aa 158-167, aa 159-168, aa 160-169, aa 158-168, aa 159-169, aa 158-169. More specifically, the invention relates to a 1,4-benzodiazepine derivatized with one of the following peptides or part thereof:

$(FAKYLWEWASVR)_2$-KKGK(bio)GA (SEQ ID NO 4)
FAKYLW-K(bio) (SEQ ID NO 5)
FAKYLWEW-K(bio) (SEQ ID NO 6)
FAKYLWEWAS-K(bio) (SEQ ID NO 7)
FAKYLWEWASVR-K(bio) (SEQ ID NO 8)
FAKYLW (SEQ ID NO 9)
FAKYLWEW (SEQ ID NO 10)
FAKYLWEWAS (SEQ ID NO 11)
FAKYLWEWASVR (SEQ ID NO 2)

Linking IGP 1362 or parts thereof to BDA 250 or BDA 452 or any derivatives thereof yields a compound with an improved specificity in blocking the interaction of annexin V with HBsAg. It should be noted that not any sequence yields the same result. For instance, the sequence presented by peptide IGP 1363, which is also derived from HBsAg (aa 115-125), does

not inhibit the binding of annexin V to HBsAg.

In another specific embodiment of the present invention, the 1,4-benzodiazepine or 1,4-benzothiazepine is derivatized with a polypeptide that consists of the following amino acid sequence or part thereof:

FARFLWEWASVR-K(bio) (SEQ ID NO 12)

FGKFLWEWASAR-K(bio) (SEQ ID NO 13)

LGKYLWEWASAR-K(bio) (SEQ ID NO 14)

FAKFLWEWASVR-K(bio) (SEQ ID NO 15)

KYGW-K(bio) (SEQ ID NO 16)

KFGW-K(bio) (SEQ ID NO 17)

RFGW-K(bio) (SEQ ID NO 18)

AYLW-K(bio) (SEQ ID NO 19)

AFLW-K(bio) (SEQ ID NO 20)

KYLW-K(bio) (SEQ ID NO 21)

RFLW-K(bio) (SEQ ID NO 22)

KFLW-K(bio) (SEQ ID NO 23)

FARFLWEWASVR (SEQ ID NO 24)

FGKFLWEWASAR (SEQ ID NO 25)

LGKYLWEWASAR (SEQ ID NO 26)

FAKFLWEWASVR (SEQ ID NO 27)

KYGW (SEQ ID NO 28)

KFGW (SEQ ID NO 29)

RFGW (SEQ ID NO 30)

AYLW (SEQ ID NO 31)

AFLW (SEQ ID NO 32)

KYLW (SEQ ID NO 33)

RFLW (SEQ ID NO 34)

KFLW (SEQ ID NO 35)

The above mentioned peptides or parts thereof can be linked to the 1,4-benzodiazepine or 1,4-benzothiazepine at positions 1, 2, 2', 3, 3' and/or 4 such as described above.

In a preferred embodiment the peptides are linked at positions 1 (R') and/or 3 (R) of the 1,4-benzodiazepine according to formula IV. The sum of both peptides (R+R') then represents a peptide as described above or part thereof. Accordingly the 1,4-benzodiazepine may take any position within the above mentioned peptides. For example, when R+R' = FAKYLWEWASVR (SEQ ID NO 2), R and/or R' may be linked to the 1,4-benzodiazepine (Bdz) in various ways:

Bdz-FAKYLWEWASVR: R' = FAKYLWEWASVR (SEQ ID NO 2); or
F-Bdz-AKYLWEWASVR: R = F, R' = AKYLWEWASVR (SEQ ID NO 36); or
FA-Bdz-KYLWEWASVR: R = FA (SEQ ID NO 37), R' = KYLWEWASVR (SEQ ID NO 38); or
FAK-Bdz-YLWEWASVR: R = FAK (SEQ ID NO 39), R' = YLWEWASVR (SEQ ID NO 40); or
FAKY-Bdz-LWEWASVR: R = FAKY (SEQ ID NO 41), R' = LWEWASVR (SEQ ID NO 42); or
FAKYL-Bdz-WEWASVR: R = FAKYL (SEQ ID NO 43); R' = WEWASVR (SEQ ID NO 44); or
FAKYLW-Bdz-EWASVR: R = FAKYLW (SEQ ID NO 9), R' = EWASVR (SEQ ID NO 45); or
FAKYLWE-Bdz-WASVR: R = FAKYLWE (SEQ ID NO 46), R' = WASVR (SEQ ID NO 47); or
FAKYLWEW-Bdz-ASVR: R = FAKYLWEW (SEQ ID NO 10), R' = ASVR(SEQ ID NO 48); or
FAKYLWEWA-Bdz-SVR: R = FAKYLWEWA (SEQ ID NO 49), R' = SVR (SEQ ID NO 50); or
FAKYLWEWAS-Bdz-VR: R = FAKYLWEWAS (SEQ ID NO 11), R' = VR (SEQ ID NO 51); or
FAKYLWEWASV-Bdz-R: R = FAKYLWEWASV (SEQ ID NO 52), R' = R; or
FAKYLWEWASVR-Bdz: R = FAKYLWEWASVR (SEQ ID NO 2);

wherein R is linked at position 3 via the C-terminus of the peptide after modification of position 3 with amine and/or R' is linked at position 1 via the N-terminus of the peptide after modification of position 1 with carboxymethyl. In case only R is a peptide, position 1 is methylated or carboxymethylated. In case only R' is a peptide, position 3 contains amine. Examples of these compounds are shown in figure 8.

The 1,4-benzodiazepine can also be linked to only part of the above mentioned peptides. For instance, the 1,4-benzodiazepine can take any position within the above mentioned peptides with deletion of one or more amino acids.

When R+R' = FAKYLWEWASVR (SEQ ID NO 2), part thereof may be linked to the 1,4-benzodiazepine (Bdz) in various ways such as for example:

Bdz-AKYLWEWASVR; or Bdz-KYLWEWASVR; or

F-Bdz-KYLWEWASVR; or
F-Bdz-YLWEWASVR; or
FA-Bdz-YLWEWASVR; or
FA-Bdz-LWEWASVR; or
FAK-Bdz-LWEWASVR; or
FAK-Bdz-WEWASVR; or
FAKY-Bdz-WEWASVR; or
FAKY-Bdz-EWASVR; or
FAKYL-Bdz-EWASVR; or
FAKYL-Bdz-WASVR; or
FAKYLW-Bdz-WASVR or;
FAKYLW-Bdz-ASVR; or
FAKYLWE-Bdz-ASVR; or
FAKYLWE-Bdz-SVR; or
FAKYLWEW-Bdz-SVR; or
FAKYLWEW-Bdz-VR; or
FAKYLWEWA-Bdz-VR; or
FAKYLWEWA-Bdz-R; or
FAKYLWEWAS-Bdz-R; or
FAKYLWEWAS-Bdz; or
FAKYLWEWASV-Bdz;

wherein the peptides are linked via their C-terminus and/or N-terminus as described above.

The peptides as described above or part thereof can also be linked to position 3 via their N-terminus by use of a linker as described above. Examples of such compounds are shown in figure 11.

The present invention further relates to a polypeptide consisting of one of the following amino acids sequences or part thereof:

FAKYLW-K(bio) (SEQ ID NO 5)
FAKYLWEW-K(bio) (SEQ ID NO 6)
FAKYLWEWAS-K(bio) (SEQ ID NO 7)
FAKYLWEWASVR-K(bio) (SEQ ID NO 8)
FARFLWEWASVR-K(bio) (SEQ ID NO 12)
FGKFLWEWASAR-K(bio) (SEQ ID NO 13)
LGKYLWEWASAR-K(bio) (SEQ ID NO 14)
FAKFLWEWASVR-K(bio) (SEQ ID NO 15)
KYGW-K(bio) (SEQ ID NO 16)
KFGW-K(bio) (SEQ ID NO 17)
RFGW-K(bio) (SEQ ID NO 18)
AYLW-K(bio) (SEQ ID NO 19)

AFLW-K(bio) (SEQ ID NO 20)

KYLW-K(bio) (SEQ ID NO 21)

RFLW-K(bio) (SEQ ID NO 22)

KFLW-K(bio) (SEQ ID NO 23)

FAKYLW (SEQ ID NO 9)

FAKYLWEW (SEQ ID NO 10)

FAKYLWEWAS (SEQ ID NO 11)

FARFLWEWASVR (SEQ ID NO 24)

FGKFLWEWASAR (SEQ ID NO 25)

LGKYLWEWASAR (SEQ ID NO 26)

FAKFLWEWASVR (SEQ ID NO 27)

KYGW (SEQ ID NO 28)

KFGW (SEQ ID NO 29)

RFGW (SEQ ID NO 30)

AYLW (SEQ ID NO 31)

AFLW (SEQ ID NO 32)

KYLW (SEQ ID NO 33)

RFLW (SEQ ID NO 34)

KFLW (SEQ ID NO 35)

These peptides are natural or homology variants of the annexin binding epitope of the HBsAg of HBV genotype A/B. Surprisingly, they were also found to inhibit the interaction between annexin V and the HBsAg of HBV.

The polypeptides used in the compounds of the present invention may consist of amino acids under the form of L, under the form of D, or under a mixed form. In a preferred embodiment, the compound of the invention is characterised that all amino acids are under the L from. In another preferred embodiment, the compound of the invention is characterised that all amino acids are under the D form.

Also included in the present invention are compounds comprising or consisting of reverso-inverso polypeptides (such as described by Guichard et al., 1994) of the polypeptides as described above.

It should also be noted that all compounds mentioned may be lead compounds, which upon additional derivation of either the 1,4-benzodiazepine/ 1,4-benzothiazepine core or the peptide ligand may be further improved.

The present invention also relates to a combination of a polypeptide as defined above and a negatively charged phospholipid such as phosphatidylserine. The interaction between phosphatidylserine and annexin was already demonstrated in WO 97/07268. The combination of said polypeptide and said negatively charged phospholipid component may be in any possible way known in the art such as for instance in the form of covalently or non-covalently coupled molecules or in the form of liposomes, etc.

The present invention further relates to a method for the production of the above mentioned 1,4-benzodiazepines or 1,4-benzothiazepines derivatives. Methods for the production of 1,4-benzodiazepines or 1,4-benzothiazepines are well known in the art. They include but are not limited to the methods described by Sternbach et al. (1963), US 3109843,US 3116203, US 3121114, US 3123529 and US 3203990. 1,4-benzodiazepines and 1,4-benzothiazepines are also commercially available from for example Sigma (Dreisenhofen, Germany) or Neosystem (Strasbourg, France). The polypeptides derived from the annexin binding proteins, more particularly, from the viral proteins that bind annexins, more particularly from the HBsAg of the HBV and/or HDV, from the glycoprotein B of the cytomegalovirus or from an annexin binding protein of any influenza virus are linked to the 1,4-benzodiazepines or 1,4-benzothiazepines by any method known in the art. For example, the peptide can be linked by Fmoc based peptide synthesis using (R,S)-Fmoc-3-amino-N-1-carboxymethyl-2-oxo-5-cyclohexyl-1,4-benzodiazepine (FB03601; Neosystem, Strasbourg, France; figure 7) as building block. Examples of the resulting molecules are shown in figure 8. Other 1,4-benzodiazepines derivatives can be synthesised by linking of a peptide via its N-terminus to position 3 of 1,4-benzodiazepine by use of a homobifunctional linker as described in the Pierce catalogue (Pierce, Rockford, IL, USA). Such reactions are shown in figures 9 or 10. Examples of the resulting molecule are shown in figure 11.

The present invention also relates to the use of the compounds of the invention for the preparation of a medicament to prevent or treat any disease in which interactions between annexin family members and annexin binding proteins are involved.

More particularly the present invention relates to the use of the compounds of the invention for the preparation of a medicament to prevent or treat any viral disease in which interactions

between viral proteins and annexin family members are involved.

More particularly, the present invention relates to the use of compounds of the invention for the preparation of a medicament to interfere with the life cycle of HBV and/or HDV by inhibiting the interaction of HBV and/or HDV with annexin V, and consequently, to prevent or treat HBV and/or HDV infections. In this regard, it should be clear that the usage of the terms "HBV and/or HDV" indicate that an infection with HBV can occur solely or can be accompanied by a superinfection with HDV. On the other hand, an infection with HDV does not occur solely. In other words, the molecules of the present invention can be used for the preparation of a medicament to prevent or treat a HBV infection solely or a mixed HBV/HDV infection.

The present invention further relates to the use of compounds of the invention for the preparation of a medicament to interfere with the life cycle of the cytomegalovirus by inhibiting the interaction of the cytomegalovirus with annexin II, and consequently, to prevent or treat cytomegalovirus infections.

The present invention further relates to the use of the compounds of the invention for the preparation of a medicament to interfere with the life cycle of influenza virus by inhibiting the interaction of influenza virus with annexin V, and consequently, to prevent or treat influenza virus infections.

Accordingly, the present invention relates to the use of the compounds of the invention for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

The present invention further relates to the use of BDA753, a compound according to formula II, or a compound according to formula III for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

In the present invention, BDA753 is specifically used to inhibit the interaction of HBV with annexin V. Although the annexin V binding of 1,4-benzodiazepines and 1,4-benzodiazepine

derivatized with AYGW has been demonstrated (Hoffman et al., 1998), 1,4-benzodiazepines without a peptide derivation or derivatized with only one amino acid at position 3 were not able to inhibit the interaction of the HBsAg of HBV to annexin V. To our surprise derivation of the 1,4-benzodiazepine with the peptide AYGW resulted in a compound that was able to inhibit the interaction of the HBsAg of HBV to annexin V.

Accordingly, the present invention also relates to the use of a peptide containing the following amino acid sequence or part thereof: AYGW (SEQ ID NO 3) for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

The present invention further relates to a pharmaceutical composition or medicament (both terms can be used interchangeably) comprising at least a compound of the invention, at least BDA 753, at least a compound according to formula II, at least a compound according to formula III or at least the peptide AYGW (SEQ ID NO 3) and possibly, a pharmaceutically acceptable carrier or excipient (both terms can be used interchangeably) to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, influenza virus infections, or infections by any strain mutated thereof. Optionally, the composition may contain other therapeutic agents useful against the above mentioned infections. These therapeutic agents include but are not limited to adefovir, BMS 200475, famciclovir, foscarnet, fiacitabine, fialuridine, (-)-FTC, ganciclivor, GEM 132, interferon, lamivudine, L-FMAU, lobucavir, n-docosanol, ribavirin, sorivudine, vidarabine or compounds mentioned in WO 98/18818 for HBV; acyclovir, adefovir, cidofovir, cyclic HPMPC, fiacitabine, fomivirsen, ganciclovir, I263W94, lobucavir, ribavirin, valaciclovir or vidarabine for cytomegalovirus; amantadine, GG167, GS4104, ribavirin or rimantadine for influenza. The "medicament" may be administered by any suitable method within the knowledge of the skilled man. Preferably, the mode of administration can be enteral or parenteral. The composition of the present invention may take the form of any of the known pharmaceutical compositions for such methods of administration. The compositions may be formulated in a manner known to those skilled in the

art, to give a controlled release, for example rapid release or sustained release of the active compound. The active substances of these pharmaceutical compositions may also be administered alone, without a carrier vehicle. However, they may also be administered with pharmaceutically acceptable non-toxic carriers or diluents, the proportions of which are determined by the suitability and chemical nature of the particular carrier. Suitable carriers or excipients known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives.

In parenteral administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. However, the dosage and mode of administration will depend on the individual. Generally, the medicament is administered so that the compound of the present invention is given at a dose between 1 μg/kg and 100 mg/kg, more preferably between 10 μg/kg and 20 mg/kg, most preferably between 0.1 and 2 mg/kg. Preferably, it is given as a bolus dose. Continuous infusion may also be used. If so, the medicament may be infused at a dose between 1 and 100 μg/kg/minute, more preferably between 5 and 20 μg/kg/minute.

Dosage forms suitable for oral administration include but are not limited to tablets, pills, capsules, caplets, granules, powders, elixirs, syrups, solutions and aqueous or oil suspensions. A suitable daily dose of the active compound for administration to human beings is generally from about 1 mg to about 5000 mg, more usually from about 5 mg to about 1000 mg, given in a single dose or in divided doses at one or more times during the day.

The present invention finally relates to the use of a compound as described above in a method to screen for molecules that block the binding between annexin and any protein which is interacting with annexin, more particularly, any viral protein which is interacting with annexin, more particularly, the HBsAg protein of HBV, glycoprotein B of cytomegalovirus or annexin binding proteins of any influenza virus. As used herein, the term "a method to screen for molecules" refers to any assay known in the art suitable for molecule screening. In particular, the term refers to the assay described in example 1 of the present invention.

The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these examples

are illustrative and can not be construed as to restrict the invention in any way.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or group of integers or steps.

BRIEF DESCRIPTION OF TABLES AND FIGURES

Table 1 provides sequence information concerning the region of amino acids 99 to 169 of "small" HBsAg that determines 6 genotypes of HBsAg (A, B ,C, D, E, F) and regarding the polypeptides which were examined for binding to annexin V.

Figure 1 shows the basic structure formula (formula I) of the 1,4-benzodiazepines and 1,4-benzothiazepines wherein:

* at position 1: -benzothiazepines have a sulphur atom (X represents S); X may also be SO_n with n = 0, 1, 2;
-benzodiazepines have a nitrogen atom (X represents N) which allows additional modifications with a peptide as defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, linked via the C-terminus of the peptide or linked to the N-terminus of the peptide after additional modification of position 1 with carboxymethyl (-CH_2COOH). In case N is not modified with a peptide, modification can be H, alkyl, phenyl, -COZ in which Z stands for H, alkyl, phenyl or substituted phenyl;

* positions 1, 2, 3, 4, 5 may form double bonds with adjacent positions; if this is the case the side chains R_2, R_4, R_5 or R_7 are non-existing;

* R_1 and/or R_2 may represent at position 2 and/or 2' H, amine, alkyl or position 2 may be oxidised (=O). In case R_1 and/or R_2 is amine, this may be further substituted with a peptide defined in the

claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, either directly linked via the C-terminus of the peptide or linked to the N-terminus of the peptide via a linker such as glutaraldehyde or succinicanhydride;

* R_3 and/or R_4 may represent at position 3 and/or 3' H, amine or alkyl. In case R_3 and/or R_4 is amine, this may be further substituted with a peptide defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein" or part thereof, either directly linked via the C-terminus of the peptide or linked to the N-terminus of the peptide via a linker such as glutaraldehyde or succinicanhydride;

* R_5 may represent at position 4 a side chain defined in the claims as "a peptide containing an annexin binding epitope of an annexin binding protein", or part thereof, either directly linked via the C-terminus of the peptide or linked to the N-terminus of the peptide after additional modification of position 4 with carboxymethyl (-CH_2COOH). In case R_5 is not a peptide as defined by the claims, R_5 may represent H, alkyl, CO-R_{12} or 3-(1-(4-benzyl)piperidinyl)propionyl);

* R_6 and/or R_7 represent H or alkyl or phenyl, possibly further substituted with alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl;

* R_8, R_9, R_{10} and/or R_{11} represent H, alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl;

* R_{12} represents H or alkyl or phenyl possibly further substituted with alkyl, cyano, halo, nitro, alkylalkoxy, alkanoyl, carboxy, alkanoylalkoxy, carbamoyl.

Figure 2 shows the structure of formula II wherein (FR 2479818):

* at position 2, R represents an amino acid or a peptide consisting of two or three amino acids;

* X represents chlorine or fluorine.

Figure 3 shows the structure of formula III wherein position 3 of the 1,4-benzodiazepine is substituted with the peptide ARPYN and position 1 is substituted with a L residue (Nachman et

al., 1998).

Figure 4 shows the structure of formula IV wherein:

* R, R' and/or R + R' constitute a peptide containing an annexin binding epitope of an annexin binding protein or part thereof. In case only R is a peptide, position 1 is methylated. In case only R' is a peptide, position 3 contains amine;

* X represents the N-terminus of the peptide which may be modified with $-COCH_3$;

* Y represents the C-terminus of the peptide which may be modified with $-NH_2$ or -Lysine-Biotine.

Figure 5 shows the structure of formula V wherein the peptide R is linked via its N-terminus by a linker (SPC).

Figure 6 shows the structures of 1,4-benzodiazepines derivatives described in example 1. BDA250: 1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one; BDA452: 3-(R,S)-(L-tryptophanyl)-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one; BDA753: 3-(R,S)-all-L-(NH-Trp-Gly-Tyr-Ala-H)-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one.

Figure 7 shows the structure of the Fmoc block (R,S)-Fmoc-3-amino-N-1-carboxymethyl-2-oxo-5-cyclohexyl-1,4-benzodiazepine (FB03601; Neosystem, Strasbourg, France) used for the synthesis of the 1,4-benzodiazepines derivatives.

Figure 8 shows some examples of 1,4-benzodiazepines derivatives according to formula IV.

Figure 9 shows the basic reaction for the synthesis of a 1,4-benzodiazepine derivative according to formula V by use of glutaraldehyde as a linker.

Figure 10 shows the basic reaction for the synthesis of a 1,4-benzodiazepine derivative according

to formula V by use of succinicanhydride as a linker.

Figure 11 shows some examples of 1,4-benzodiazepines derivatives according to formula V.

Figure 12 demonstrates the differential inhibition of the interaction between annexin V and HBsAg by different compounds: BDA250, BDA452 and BDA753 are described in figure 6; IGP1362 and IGP1363 are peptides derived from respectively amino acid positions 158-169 and amino acid positions 115-125 of the HBsAg of HBV and are shown in table 1. BDA 753 was able to inhibit the interaction of annexin V with HBsAg while a compound containing only the benzodiazepine part of the molecule (BDA250) did not.

Figure 13 demonstrates the differential inhibition of the interaction between annexin V and HBsAg by different peptides representing natural variants of the amino acids 158-169 of HBsAg or representing a peptide without additional modification of the C-terminus with a biotinylated lysine (IGP 1623) (table 1). The results have been normalised to the inhibition potency of IGP 1481.

Figure 14 demonstrates the differential inhibition of the interaction between annexin V and HBsAg by different peptides representing C-terminal truncated variants of IGP 1481 (table 1). The results have been normalised to the inhibition potency of IGP 1481.

Figure 15 demonstrates the differential inhibition of the interaction between annexin V and HBsAg by different peptides representing non-natural mutants of IGP 1481 (table 1). The results have been normalised to the inhibition potency of IGP 1481. Peptides marked with an asterisks showed no inhibition at the highest concentration tested, in theses cases the bars represent the highest relative concentration tested.

EXAMPLES

Example 1: Influence of 1,4 benzodiazepine and derivatives on the binding of annexin V to HBsAg

To demonstrate the effect of annexin V binding molecules on the interaction of HBsAg with annexin V, a competition experiment was performed. The annexin V binding compounds (figure 6 and table 1) were allowed to compete with HBsAg in binding annexin V. Finally labelled annexin V bound to HBsAg was measured. Briefly, recombinant HBsAg was coated on microtiterplates (2 µg/ml, overnight 4°C in TBS, supplemented with 1mM $CaCl_2$ and 1mM $MgCl_2$). After blocking the plates with 3% cold fish gelatin (2h, RT in TBS, supplemented with 1mM $CaCl_2$ and 1mM $MgCl_2$), ^{125}I-labelled annexin V (50ng) was added in the presence of an excess of peptide or compound and allowed to interact (2h, 37°C, in TBS, supplemented with 1mM $CaCl_2$ and 1mM $MgCl_2$ and 0.3% cold fish gelatine). After this incubation the plates were washed three times with TBS, supplemented with 1mM $CaCl_2$ and 1mM $MgCl_2$ and 0.05% Tween-20. The experiment was conducted in six-fold and the results expressed are mean values (standard deviation is presented by lines on top of the mean). The molar excess, versus HBsAg, for each compound or peptide used is as follows:

BDA 250	25000
BDA 452	13750
BDA 753	1600
IGP 1362	400
IGP 1363	560

As can be judged from figure 12, the binding of annexin V to HBsAg was abolished by the molecules BDA 753 and IGP 1362. BDA 753 is composed of a 1,4-benzodiazepine derivatized with a peptide chain of 4 amino acids. The benzodiazepine part is equal to BDA 250, which binds annexins but was not able to inhibit the binding of HBsAg to annexin V. On the contrary, BDA250 rather enhanced binding. The molecule BDA 452 which has a similar core structure as BDA 250 but which carries an aminated tryptophan did not influence the binding of HBsAg to annexin V at all, while the molecule BDA 753 did inhibit the binding of HBsAg to annexin V. From these results it may be concluded that the three additional amino acids present on BDA 753 and not present on BDA 452 were responsible for the observed competition. Most

likely these amino acids bound at the same site on annexin V as did HBsAg. The aminated tryptophan residue already present in BDA 452 probably also interfered partially with the HBsAg binding since the additional tryptophan residue did abolish the binding enhancement caused by BDA 250. This indicates that also BDA 452 may be further modified to obtain a better specificity to inhibit the binding of annexin V to HBsAg. The peptide IGP1362, which contains aa 158-169 of HBsAg, was also able to inhibit binding of HBsAg to annexin V (figure 12) and most likely binds to the same site as does BDA 753. It should be noted that not any peptide sequence yields the same result as the sequence presented by peptide IGP1363, which is also derived from HBsAg (amino acids 115-125), did not inhibit the binding of annexin V to HBsAg. It may be concluded that the sequences present in this peptide did not bind to the HBsAg binding domain of annexin V.

Therefore, IGP1362 or parts thereof are linked to 1,4-benzodiazepines, BDA250 or BDA452 or any derivative thereof. Such molecules can be made by Fmoc based peptide synthesis using (R,S)-Fmoc-3-amino-N-1-carboxymethyl-2-oxo-5-cyclohexyl-1,4-benzodiazepine (FB03601; Neosystem, Strasbourg, France; figure 7) as building block. Examples of the resulting molecule are shown in figure 8. Other 1,4-benzodiazepines derivatives are synthesised by linking a peptide to 3 amine-1,4-benzodiazepine via its N-terminus by use of a linker molecule in a reaction as shown in figures 9 and 10. Examples of the resulting molecule are shown in figure 11. The resulting 1,4-benzodiazepines derivatives are tested in the competition experiment to demonstrate their improved specificity in blocking the interaction of annexin V with HBsAg.

Example 2: Further identification of HBsAg sequences binding to Annexin V

In order to study in more detail the inhibition of the HBsAg-Annexin V binding by peptide sequences, the binding assay was further optimised. This resulted in the following assay setup:

- coating of HBsAg to microtiterplates: 500 ng/100 µl, 1h, 37°C;
- blocking: TBS 3% (v/v) cold fish gelatin, 1h, RT;
- binding: 50 ng radiolabelled annexin V/100 µl (pre-incubated with competing compound during 1h at 37°C in TBS, 10 mM $CaCl_2$, 0.3% (v/v) cold fish gelatin), 1h, 37°C;
- washing: three times with 200 µl TBS, 10 mM $CaCl_2$, 0.05% (v/v) Tween-20, RT;
- elution of bound label with SDS (60 µl, 1h, 37°C), followed by counting in a gamma counter.

The peptide IGP 1362 is a branched peptide presenting two branches of the amino acids 158-169 of small HBsAg on a lysine core which is biotinylated (table 1). This sequence was also synthesised in a monomeric form with a C-terminal lysine carrying a biotin on the epsilon amine function, or with an amidated C-terminus (IGP 1481 and 1623, see table 1). In addition peptides were made representing the same domain of HBsAg (amino acids 158-169) but derived from other genotypes of HBV: IGP 1624 and 1625 representing the major sequences found in genotype C, IGP 1618 representing the major sequence found in genotypes D/E and IGP 1619 representing the major sequence found in genotype F, while IGP 1481 represents the major sequence found in genotypes A/B (table 1). In total the sequences of the peptides IGP 1481, 1624, 1625, 1618 and 1619 represent 90% of 460 HBsAg sequences available on public sequence databanks in October 1998. All peptides were purified by reversed phase chromatography and fractions containing peptide were analysed by mass spectrometry in order to confirm the presence of the desired peptide. The purified peptides were assayed in the annexin V/HBsAg binding assay and the concentration resulting in a 50% competition was calculated as molar excess to the coated recombinant HBsAg. The molar excess to reach the 50% competition level is 17.5 for IGP 1481, which is a sequence homologous to the coated HBsAg. The results of the other peptides are shown in figure 13 and have been normalised to the value obtained with IGP 1481 (molar excess of IGP 1481 to reach 50% competition is set to 1). As can be judged from this figure all peptides derived from other genotypes show a level of competition which is in the same order of magnitude (maximum difference is 5 times lower for IGP 1625). From this result it can be suspected that all natural variants of HBsAg existing show a similar potency. Surprisingly the peptide 1623 which is identical to 1481 except for the biotinylated lysine is 7 times less active. Thus the addition of a biotinylated lysine to the peptide alters its properties in such a way that the potency increases. IGP1481, 1618, 1619, 1624 or 1625 or parts thereof are linked to 1,4-benzodiazepine, BDA250, BDA452 or any derivatives thereof. The resulting 1,4-benzodiazepines derivatives are tested in the competition experiment to demonstrate their improved specificity in blocking the interaction of annexin V with HBsAg.

Example 3: Further delineation of HBsAg sequences binding to Annexin V

In order to determine which part of the sequence 158-169 is important for the observed

competition, C-terminal truncated peptides were synthesised, purified by reversed phase chromatography and their structure confirmed by mass spectrometry. Three such peptides, IGP 1556, 1557 and 1558 with respectively 2, 4 and 6 amino acids deleted compared to IGP 1481 (see table 1) were generated and tested for competition. The peptide IGP 1556 only lacking the 2 C-terminal amino acids is only 10 times less potent, the same is true for IGP 1557 which lacks 4 amino acids, while IGP 1558, lacking 6 amino acids is 100 times less active (figure 14).
IGP1556, 1557 or 1558 or parts thereof are linked to 1,4-benzodiazepines, BDA250, BDA452 or any derivatives thereof. The resulting 1,4-benzodiazepines derivatives are tested in the competition experiment to demonstrate their improved specificity in blocking the interaction of annexin V with HBsAg.

Example 4: Identification of compounds with improved specificity in blocking the interaction of annexin V with HBsAg.

Both the peptide IGP 1623 and BDA 753 do inhibit the interaction of annexin V with HBsAg. These compounds show however some sequence homology:

FAKYLWEWASVR
: :
AYGW-Bdz

By increasing the homology between BDA 753 and IGP 1623 or natural variants thereof such as represented by IGP 1618, 1619, 1624 and 1625, new compounds are generated with an improved specificity in blocking the interaction between annexin V and HBsAg. Such compounds are:

KYGW-Bdz
KFGW-Bdz
RFGW-Bdz
AYLW-Bdz
AFLW-Bdz
KYLW-Bdz

RFLW-Bdz

KFLW-Bdz

Bdz stands for the compound BDA250 (figure 6) in which position 3 is modified with an amine to which the peptides are coupled via their C-terminus and position 1 may be modified to carboxymethyl.

Example 5: Identification of amino acids in the region 158-169 of HBsAg of HBV, important for high specificity in blocking the interaction of annexin V with HBsAg.

In order to identify residues in the amino acid sequence 158-169 of HBsAg of HBV, important for the potency of the peptide IGP 1481 to inhibit the interaction between HBsAg and annexin V, several amino acids were mutated either to alanine or glutamine (IGP 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1493 and 1622, see table 1). All peptides were again purified by reversed phase chromatography and the presence of the desired structure confirmed by mass spectrometry. Figure 15 shows the relative competition to IGP 1481 of these peptides. These results confirm that the amino acid at position 160 is important and mutation of this lysine to alanine (IGP 1484) reduces the activity at least 20 fold (the highest concentration tested did not yet result in competition). The influence of the mutation of the amino acid alanine to lysine (or arginine) in the compound BDA 753, as specified in example 4, is thus supported by this experiment. The same observation is made for the amino acid in position 162 which is normally a leucine. Mutation of this leucine to glutamine (IGP 1486) almost abolished activity while mutation of this leucine to alanine (IGP 1622) resulted in a reduction of the activity by at least 25 fold (the highest concentration tested did not yet result in competition). The influence of the mutation of the amino acid glycine in the compound BDA 753 to leucine, as specified in example 4, is thus supported by these experiments.

Table 1: Most common sequence of HBV genotypes and polypeptides that were examined for binding to annexin V.

	100 110 120 130 140 150 160 170	
geno	* * * * * * * *	
HBsAg A	DYQGMLPVCPLIPGSTTTSTGPCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLWEWASVR	(SEQ ID NO 53)
HBsAg B	DYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLWEWASVR	(SEQ ID NO 54)
HBsAg C	DYQGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVR	(SEQ ID NO 55)
HBsAg C	K	(SEQ ID NO 56)
HBsAg D	DYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASAR	(SEQ ID NO 57)
HBsAg E	DYQGMLPVCPLIPGSSTTSTGPCRTCTTLAQGTSMYPSCCCSKPSDGNCTCIPIPSSWAFGKFLWEWASAR	(SEQ ID NO 58)
HBsAg F	DYQGMLPVCPLIPGSTTTSTGPCKTCTTLAQGTSMYPSCCCSKPSDGNCTCIPIPSSWALGKYLWEWASAR	(SEQ ID NO 59)

IGP 1363	$(TTSTGPCKTCT)_2$-KKGK(bio)GA (SEQ ID NO 60)
IGP 1362	$(FAKYLWEWASVR)_2$-KKGK(bio)GA (SEQ ID NO 4)
IGP 1481	FAKYLWEWASVR-K(bio) (SEQ ID NO 8)
IGP 1623	FAKYLWEWASVR (SEQ ID NO 2)
IGP 1624	FARFLWEWASVR-K(bio) (SEQ ID NO 12)
IGP 1618	FGKFLWEWASAR-K(bio) (SEQ ID NO 13)
IGP 1619	LGKYLWEWASAR-K(bio) (SEQ ID NO 14)
IGP 1625	FAKFLWEWASVR-K(bio) (SEQ ID NO 15)
IGP 1556	FAKYLWEWAS-K(bio) (SEQ ID NO 7)
IGP 1557	FAKYLWEW-K(bio) (SEQ ID NO 6)
IGP 1558	FAKYLW-K(bio) (SEQ ID NO 5)
IGP 1482	AAKYLWEWASVR-K(bio) (SEQ ID NO 61)

IGP 1483	FNKYLWEWASVR-K(bio)	(SEQ ID NO 62)
IGP 1484	FAAYLWEWASVR-K(bio)	(SEQ ID NO 63)
IGP 1485	FAKALWEWASVR-K(bio)	(SEQ ID NO 64)
IGP 1486	FAKYNWEWASVR-K(bio)	(SEQ ID NO 65)
IGP 1487	FAKYLAEWASVR-K(bio)	(SEQ ID NO 66)
IGP 1488	FAKYLWAWASVR-K(bio)	(SEQ ID NO 67)
IGP 1489	FAKYLWEAASVR-K(bio)	(SEQ ID NO 68)
IGP 1493	FAKYLWEWASVA-K(bio)	(SEQ ID NO 69)
IGP 1622	FAKYAWEWASVR-K(bio)	(SEQ ID NO 70)

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CLAIMS

1. A compound according to formula I derivatized with one or more peptides containing an annexin binding epitope of an annexin binding protein or part thereof, provided that said compound is not BDA753, a compound according to formula II or a compound according to formula III.

2. A compound according to formula IV derivatized with one or two peptides R and/or R', such that R, R' and/or R + R', represent a peptide containing an annexin binding epitope of an annexin binding protein or part thereof, provided that said compound is not BDA753 or a compound according to formula III.

3. A compound according to formula V derivatized with a peptide, R, containing an annexin binding epitope of an annexin binding protein or part thereof.

4. A compound according to any of claims 1 to 3 in which the annexin binding protein is a viral protein.

5. A compound according to claim 4 in which the viral protein is the surface antigen of HBV.

6. A compound according to claim 4 in which the viral protein is the glycoprotein B of the cytomegalovirus

7. A compound according to claim 4 in which the viral protein is a protein derived from any influenza strain on the condition that this influenza protein binds annexin V.

8. A compound according to claim 4 derivatized with one or more of the following peptides or part thereof:

KTCTTPAQGN (SEQ ID NO 1)

$(FAKYLWEWASVR)_2$-KKGK(bio)GA (SEQ ID NO 4)

FAKYLW-K(bio) (SEQ ID NO 5)

FAKYLWEW-K(bio) (SEQ ID NO 6)

FAKYLWEWAS-K(bio) (SEQ ID NO 7)

FAKYLWEWASVR-K(bio) (SEQ ID NO 8)

FAKYLW (SEQ ID NO 9)

FAKYLWEW (SEQ ID NO 10)

FAKYLWEWAS (SEQ ID NO 11)

FAKYLWEWASVR (SEQ ID NO 2)

FARFLWEWASVR-K(bio) (SEQ ID NO 12)

FGKFLWEWASAR-K(bio) (SEQ ID NO 13)

LGKYLWEWASAR-K(bio) (SEQ ID NO 14)

FAKFLWEWASVR-K(bio) (SEQ ID NO 15)

KYGW-K(bio) (SEQ ID NO 16)

KFGW-K(bio) (SEQ ID NO 17)

RFGW-K(bio) (SEQ ID NO 18)

AYLW-K(bio) (SEQ ID NO 19)

AFLW-K(bio) (SEQ ID NO 20)

KYLW-K(bio) (SEQ ID NO 21)

RFLW-K(bio) (SEQ ID NO 22)

KFLW-K(bio) (SEQ ID NO 23)

FARFLWEWASVR (SEQ ID NO 24)

FGKFLWEWASAR (SEQ ID NO 25)

LGKYLWEWASAR (SEQ ID NO 26)

FAKFLWEWASVR (SEQ ID NO 27)

KYGW (SEQ ID NO 28)

KFGW (SEQ ID NO 29)

RFGW (SEQ ID NO 30)

AYLW (SEQ ID NO 31)

AFLW (SEQ ID NO 32)

KYLW (SEQ ID NO 33)

RFLW (SEQ ID NO 34)

KFLW (SEQ ID NO 35)

9. A polypeptide having one of the following amino acid sequences or part thereof:

FAKYLW-K(bio) (SEQ ID NO 5)
FAKYLWEW-K(bio) (SEQ ID NO 6)
FAKYLWEWAS-K(bio) (SEQ ID NO 7)
FAKYLWEWASVR-K(bio) (SEQ ID NO 8)
FARFLWEWASVR-K(bio) (SEQ ID NO 12)
FGKFLWEWASAR-K(bio) (SEQ ID NO 13)
LGKYLWEWASAR-K(bio) (SEQ ID NO 14)
FAKFLWEWASVR-K(bio) (SEQ ID NO 15)
KYGW-K(bio) (SEQ ID NO 16)
KFGW-K(bio) (SEQ ID NO 17)
RFGW-K(bio) (SEQ ID NO 18)
AYLW-K(bio) (SEQ ID NO 19)
AFLW-K(bio) (SEQ ID NO 20)
KYLW-K(bio) (SEQ ID NO 21)
RFLW-K(bio) (SEQ ID NO 22)
KFLW-K(bio) (SEQ ID NO 23)
FAKYLW (SEQ ID NO 9)
FAKYLWEW (SEQ ID NO 10)
FAKYLWEWAS (SEQ ID NO 11)
FARFLWEWASVR (SEQ ID NO 24)
FGKFLWEWASAR (SEQ ID NO 25)
LGKYLWEWASAR (SEQ ID NO 26)
FAKFLWEWASVR (SEQ ID NO 27)
KYGW (SEQ ID NO 28)
KFGW (SEQ ID NO 29)
RFGW (SEQ ID NO 30)
AYLW (SEQ ID NO 31)

AFLW (SEQ ID NO 32)

KYLW (SEQ ID NO 33)

RFLW (SEQ ID NO 34)

KFLW (SEQ ID NO 35)

10. A compound according to any of claims 1 to 9 comprising or consisting of a polypeptide in which all amino acids are under the L form or in which all amino acids are under the D form.

11. A compound according to any of claims 1 to 10 comprising or consisting of a reverso-inverso polypeptide.

12. A method for the production of a specific molecule according to any of claims 1 to 11.

13. The use of the molecules according to any of claims 1 to 11 for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

14. The use of BDA753, a compound according to formula II, or a compound according to formula III, for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

15. The use of a peptide containing the following amino acid sequence or part thereof: AYGW (SEQ ID NO 3) for the preparation of a medicament to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family

members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

16. A pharmaceutical composition containing at least one molecule according to claims 1 to 11 or 13 to 15 to prevent or treat any disease in which protein interactions with annexin family members are involved, more particularly to prevent or treat any viral disease in which interactions between viral proteins and host annexin family members are involved, more particularly to prevent or treat HBV and/or HDV infections, cytomegalovirus infections, or influenza virus infections.

17. The use of a compound according to claims 1 to 11 or 13 to15 in a method to screen for molecules that block the binding between annexin and a protein which is interacting with annexin, more particularly, a viral protein which is interacting with annexin, more particularly, the HBsAg protein of HBV, glycoprotein B of cytomegalovirus or annexin binding proteins of any influenza virus.

FORMULA I

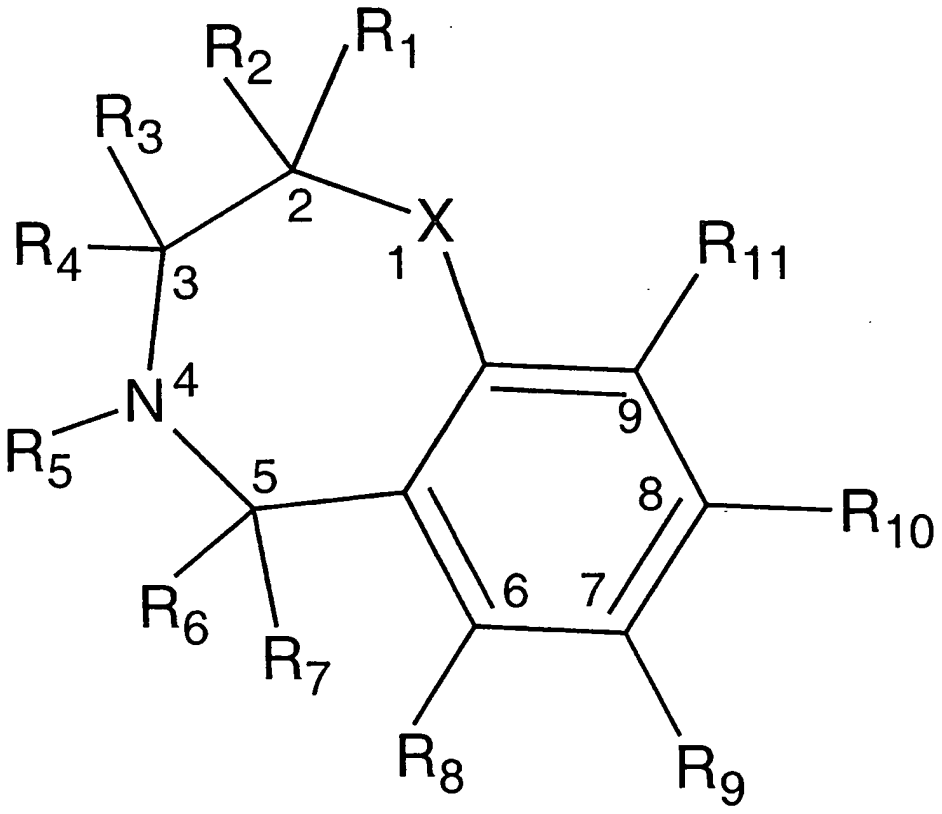

Figure 1

FORMULA II

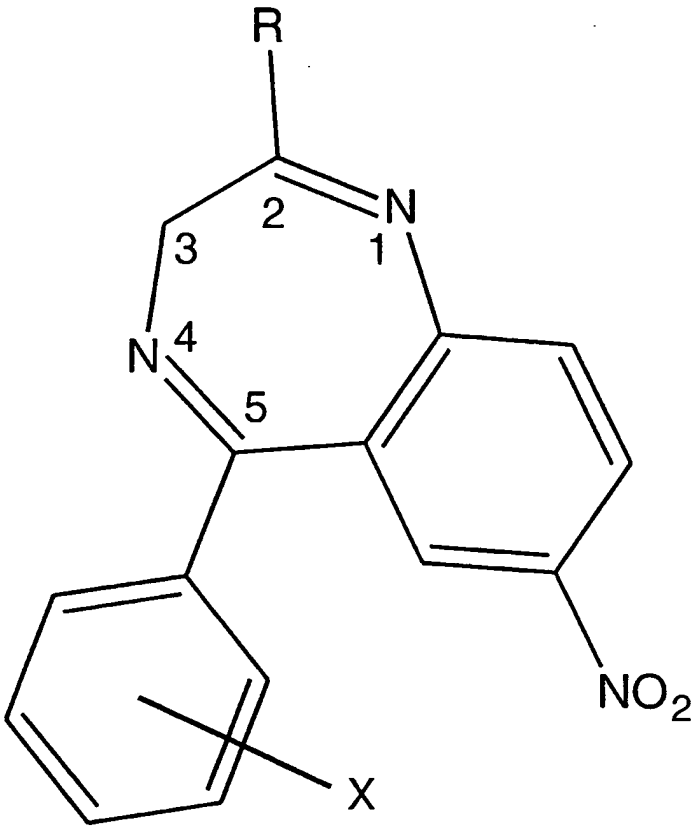

Figure 2

FORMULA III

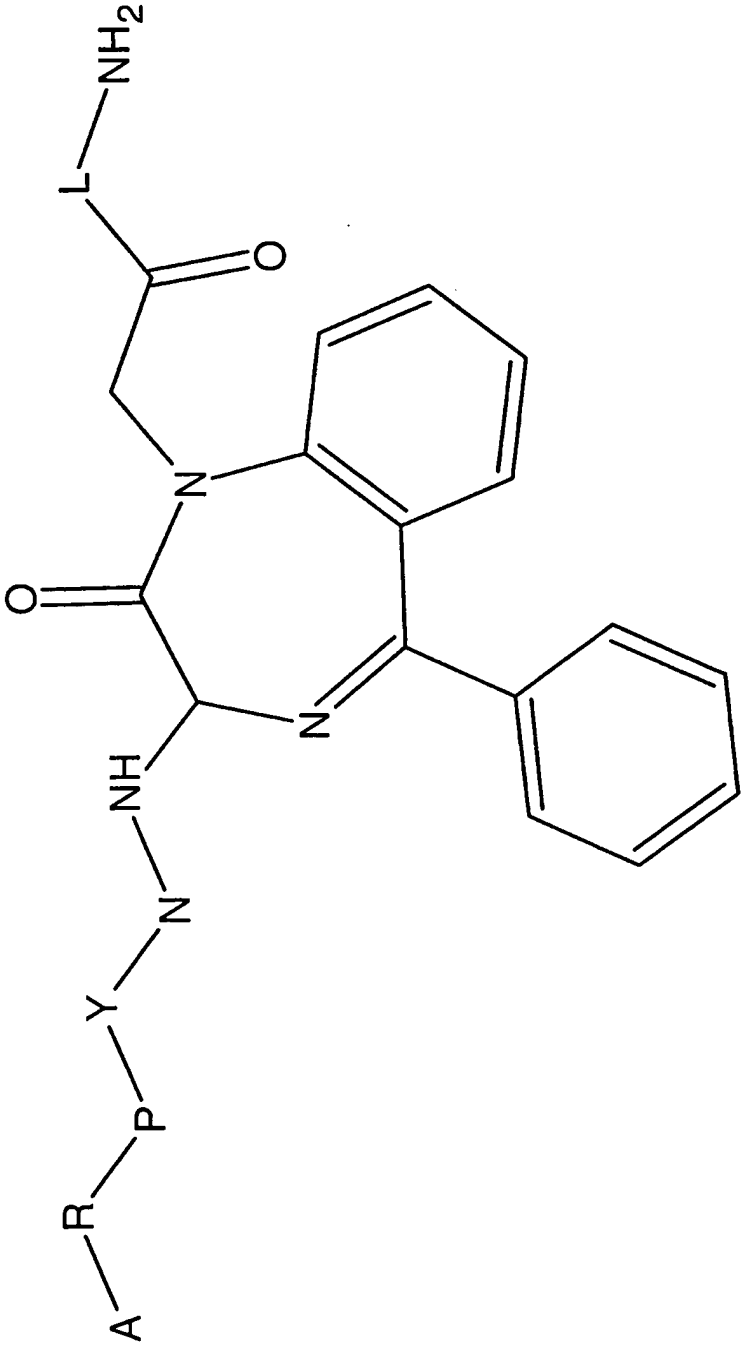

Figure 3

FORMULA IV

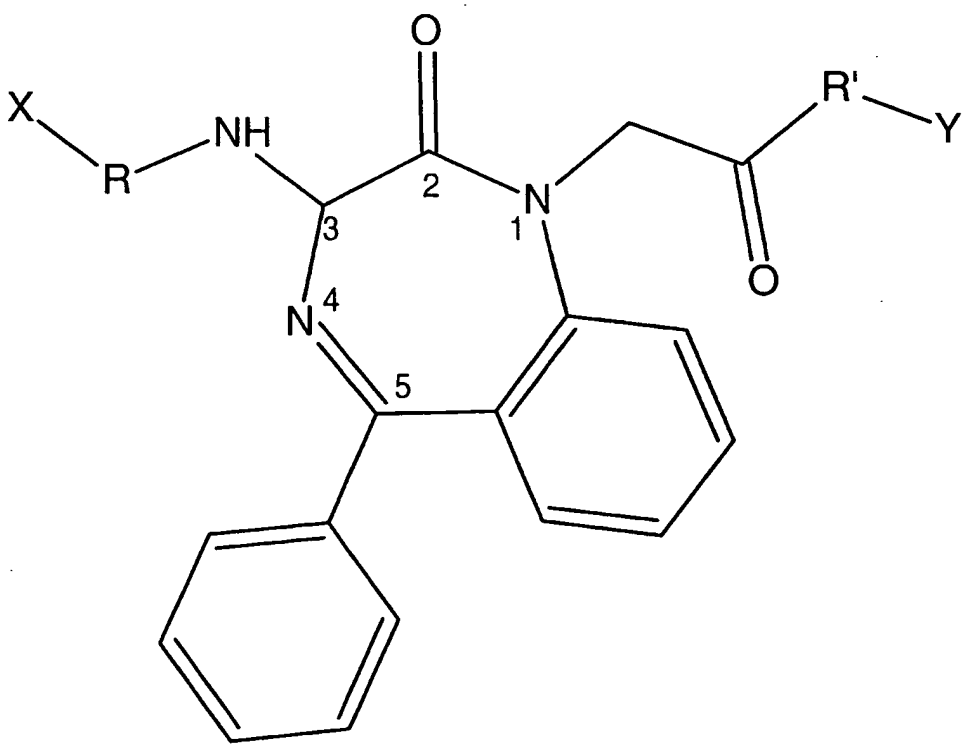

Figure 4

FORMULA V

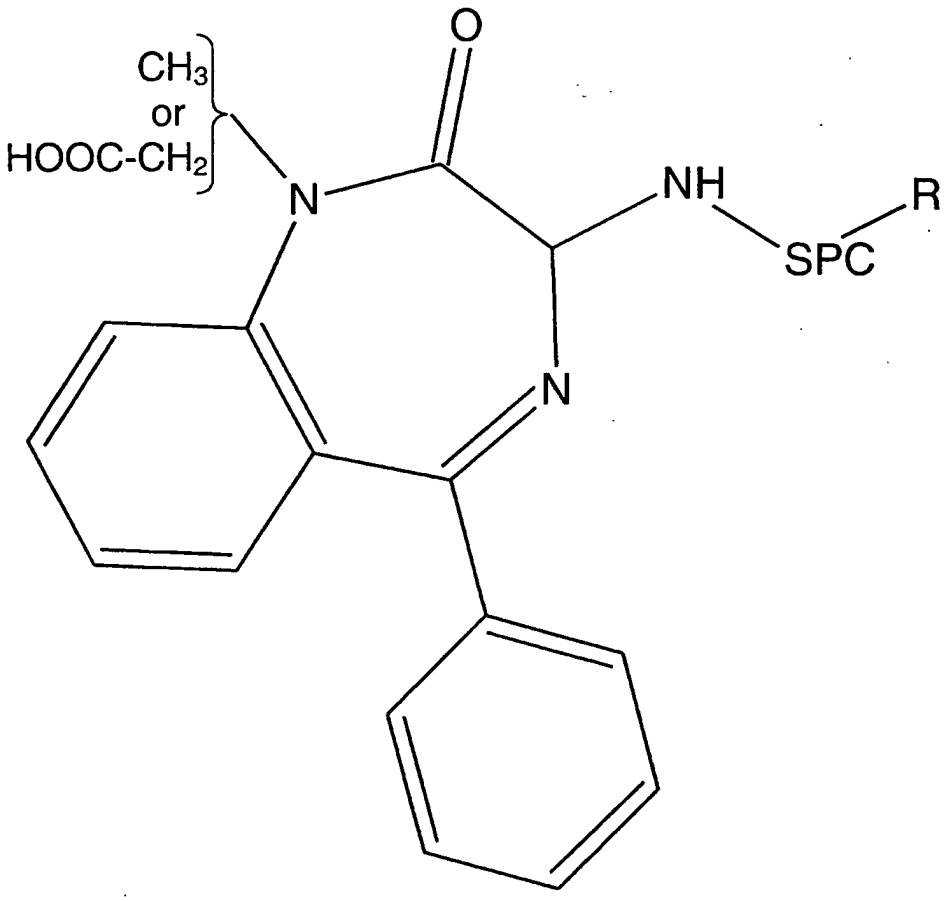

Figure 5

BDA250

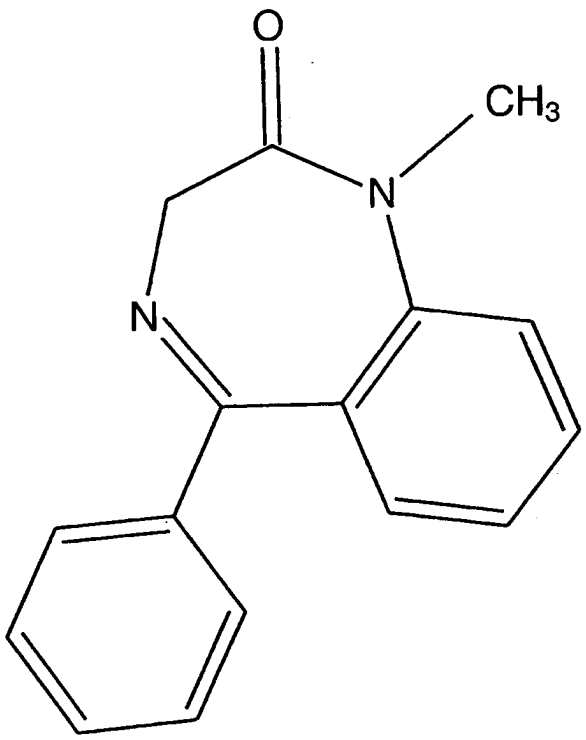

Figure 6

BDA452

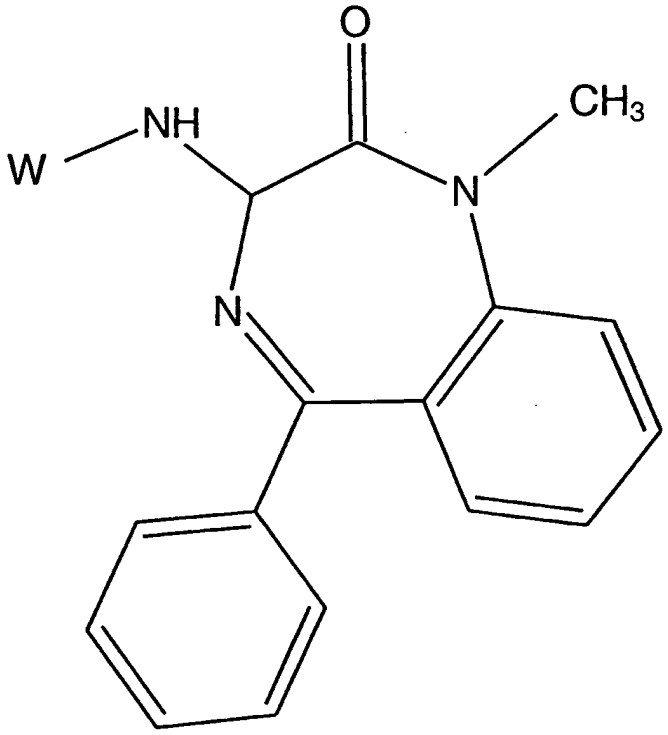

Figure 6 cont.

BDA753

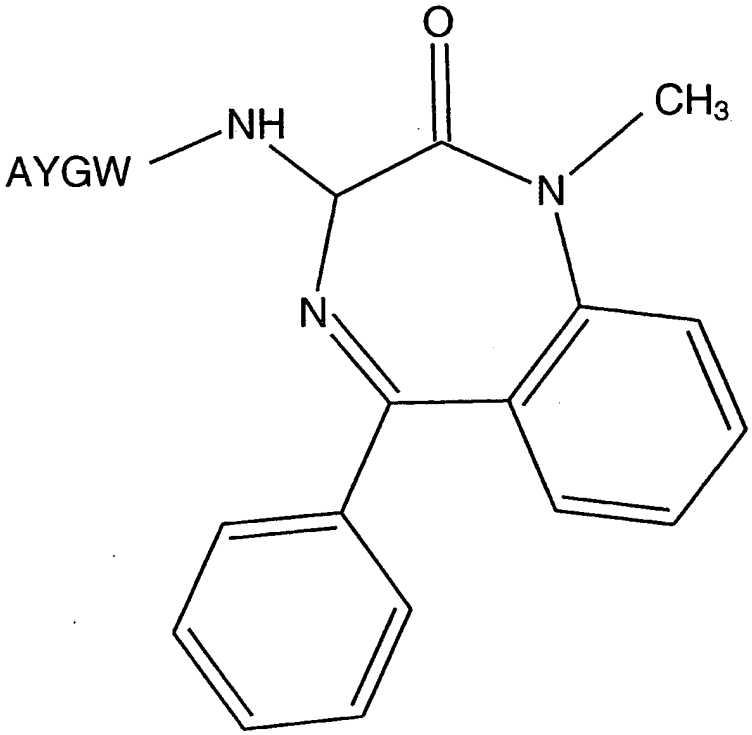

Figure 6 cont.'

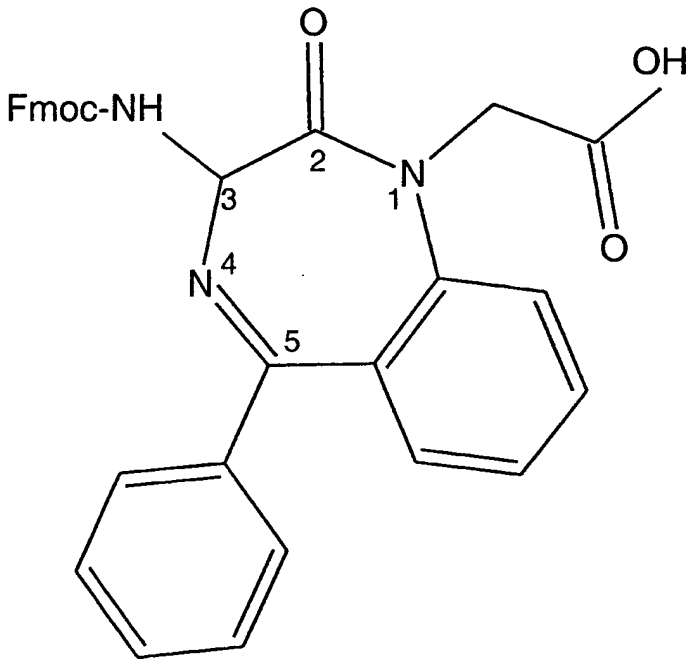

Figure 7

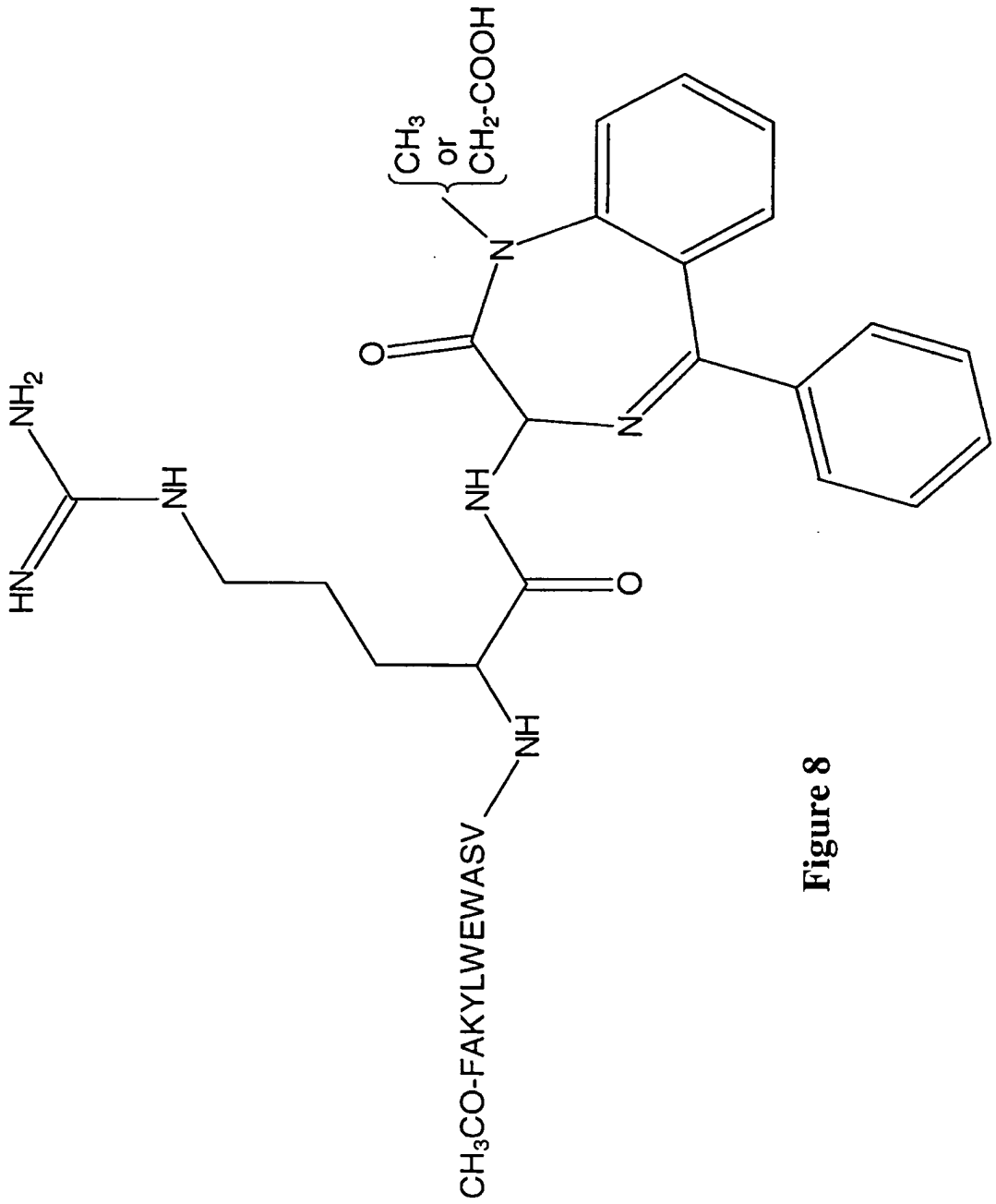

Figure 8

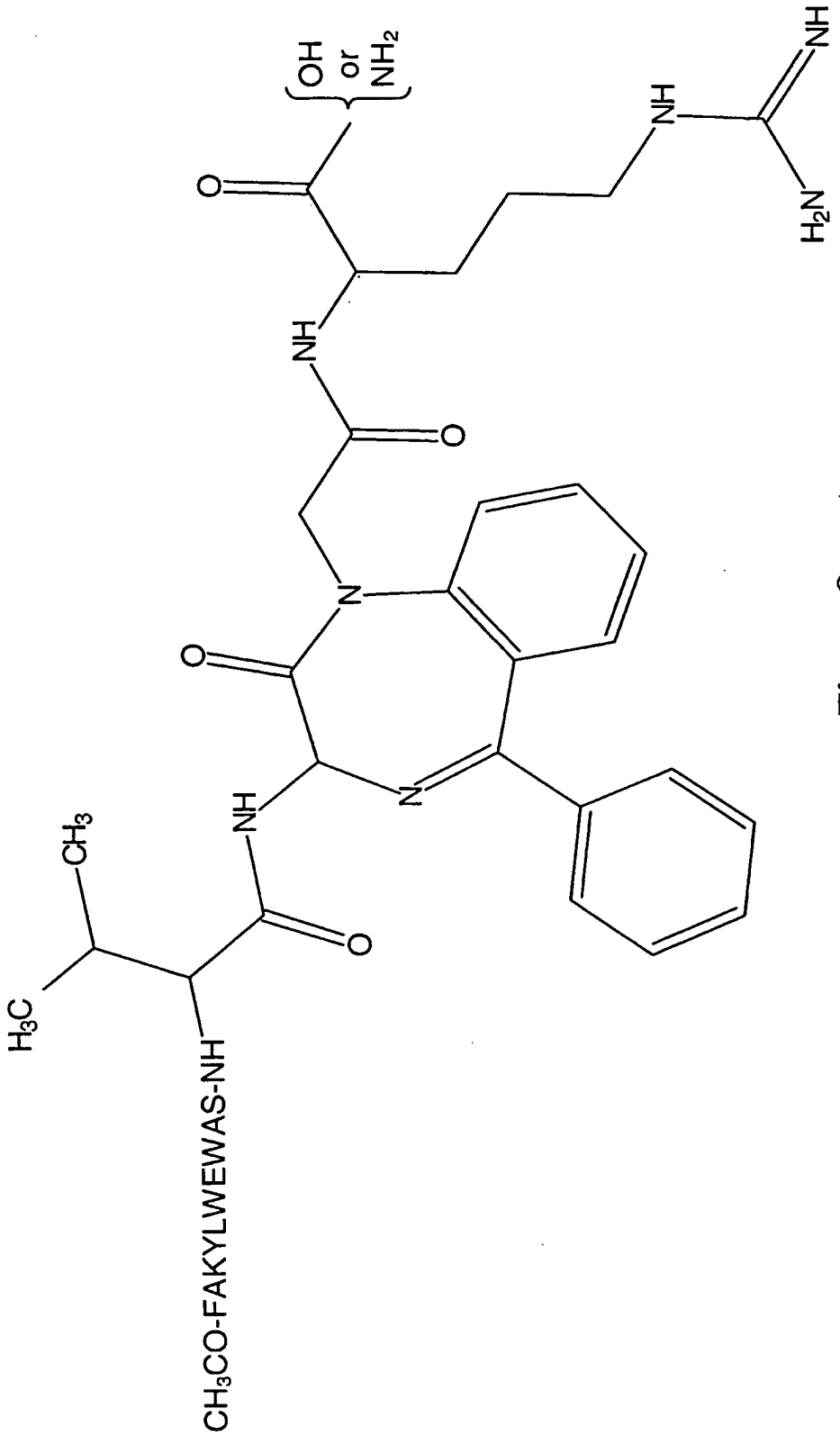

Figure 8 cont.

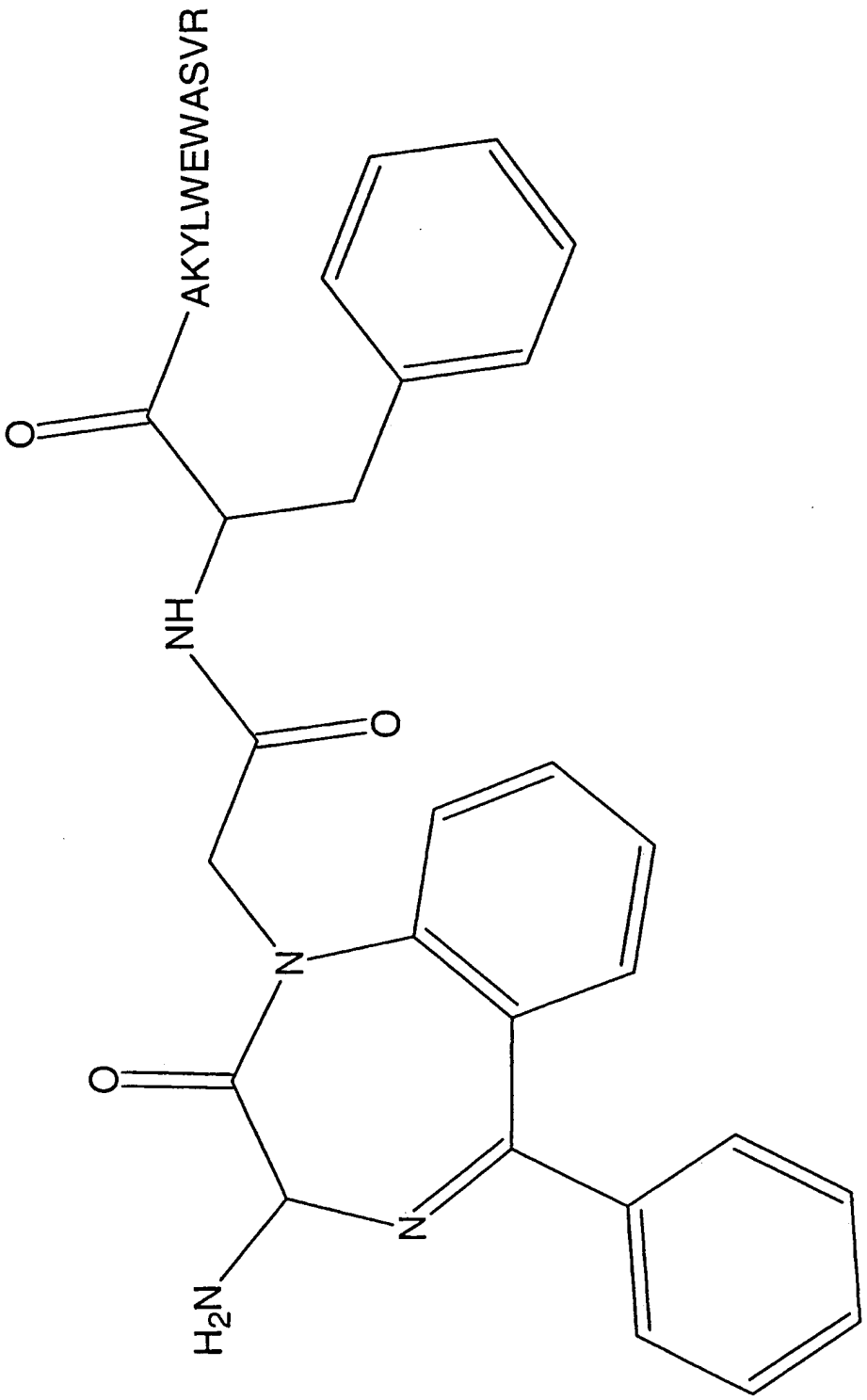

Figure 8 cont.'

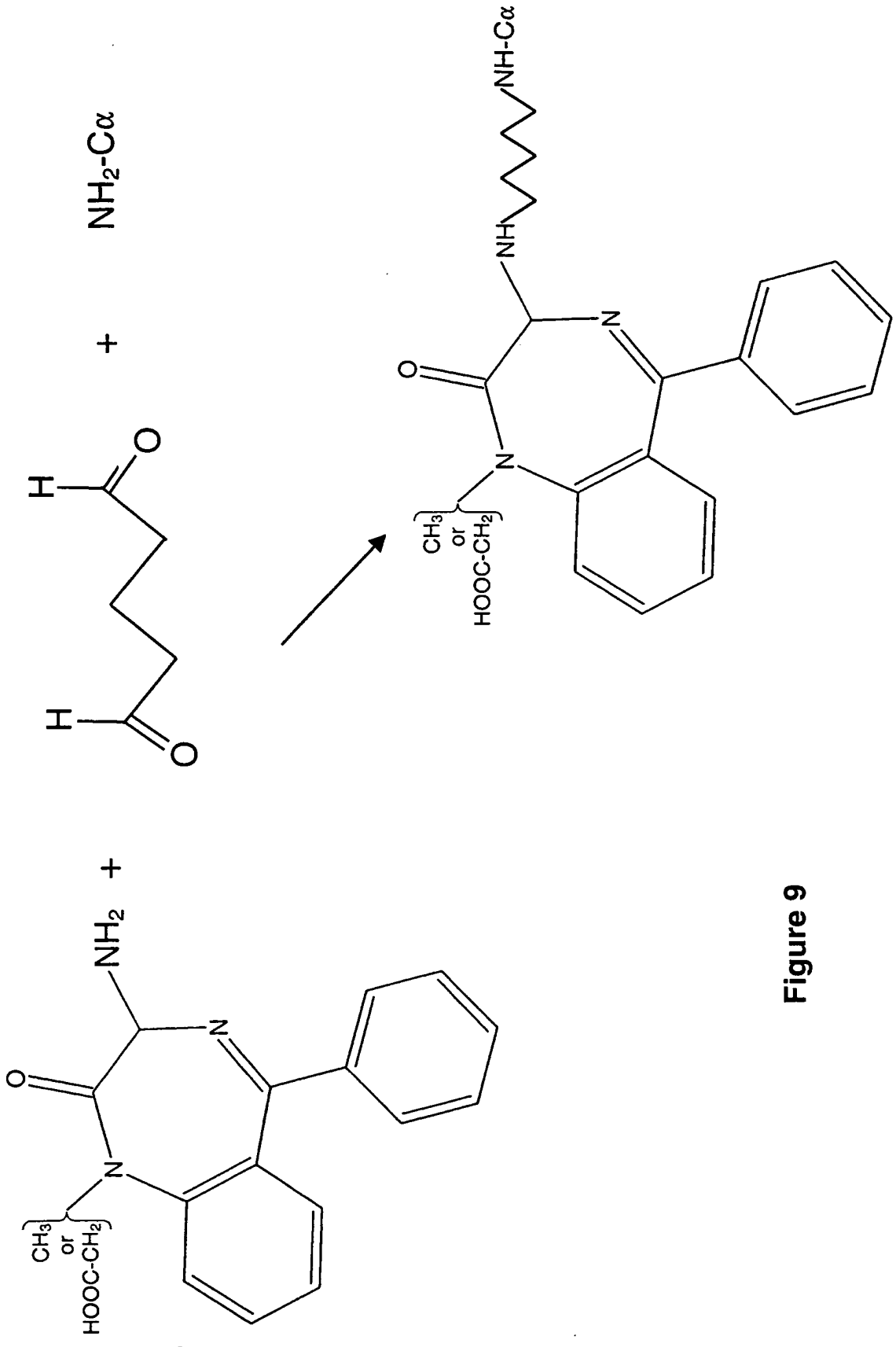

Figure 9

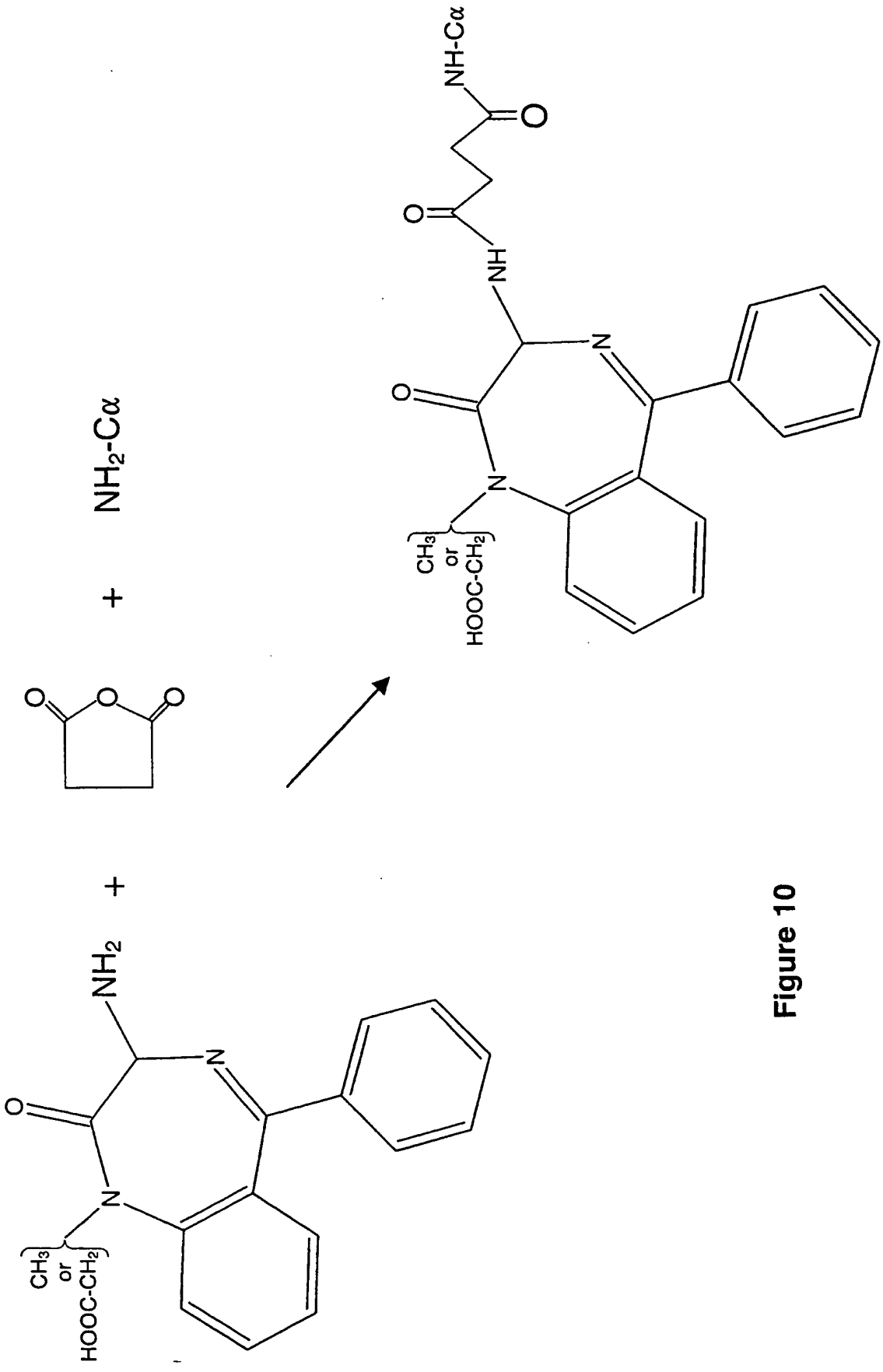

Figure 10

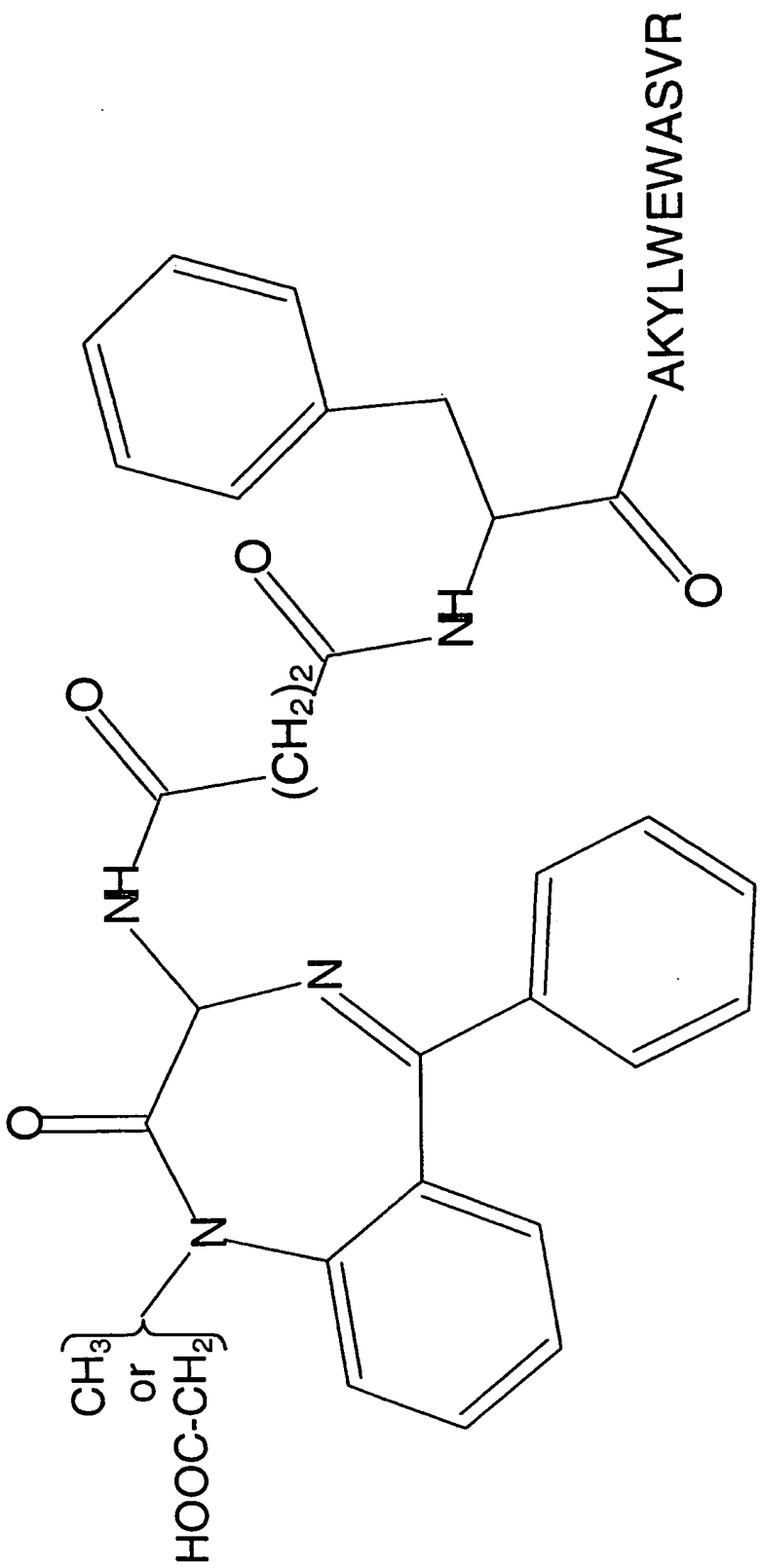

Figure 11

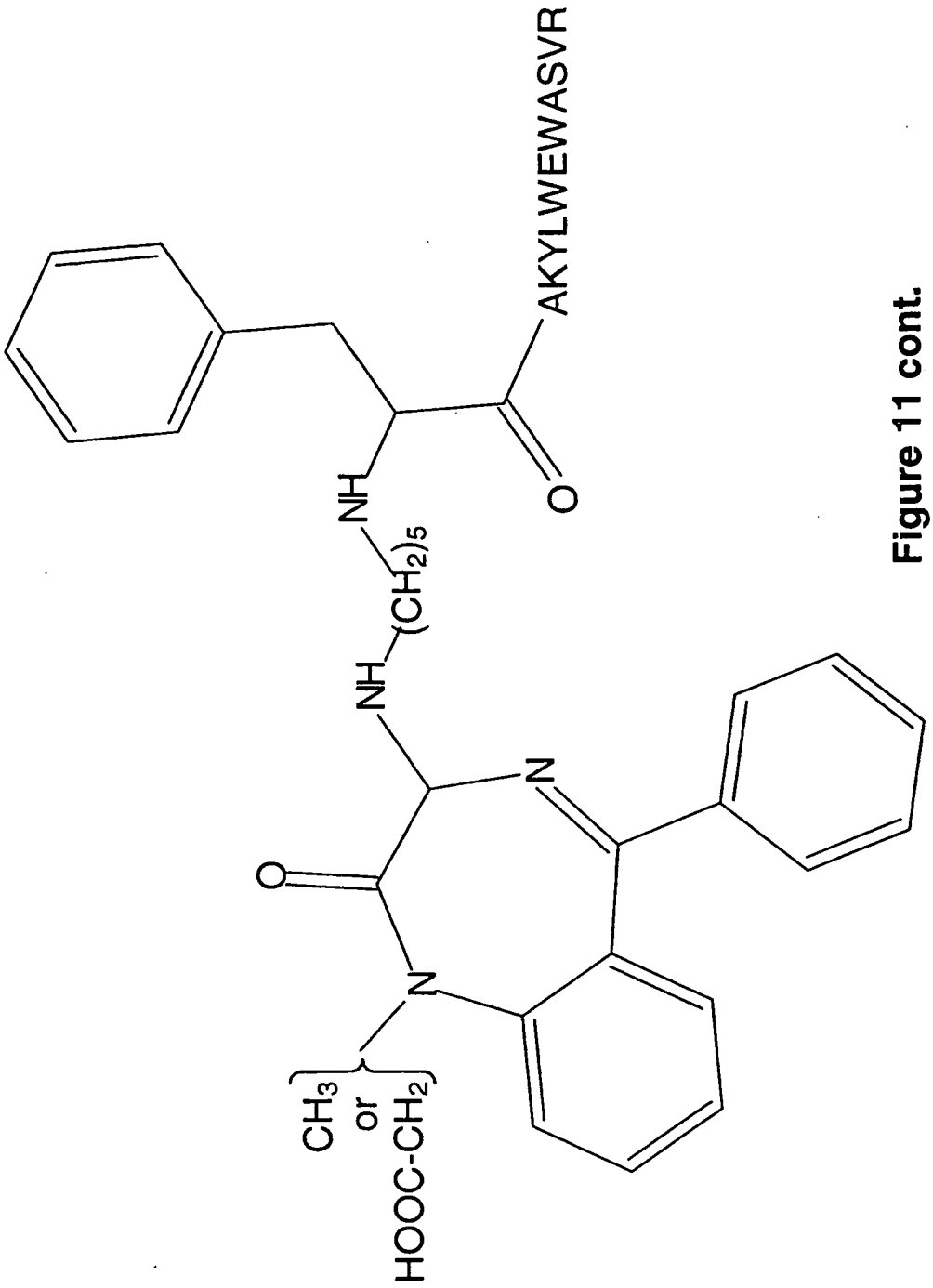

Figure 11 cont.

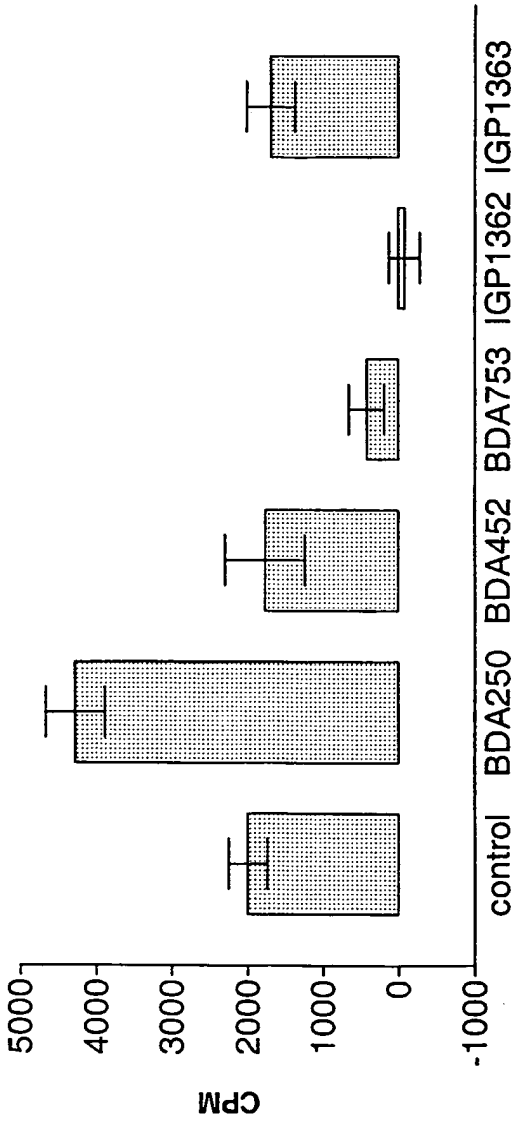
CPM
5000
4000
3000
2000
1000
0
-1000
control
BDA250
BDA452
BDA753
IGP1362
IGP1363

Figure 12

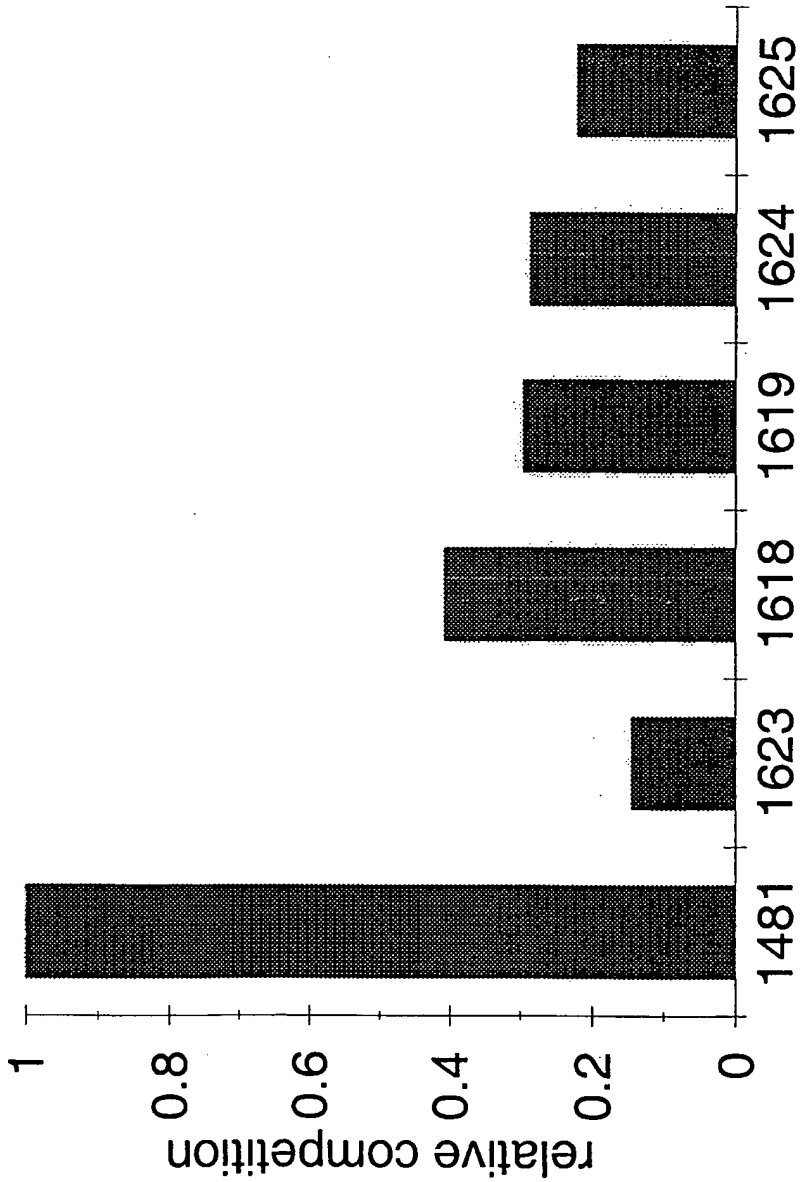
relative competition
0
0.2
0.4
0.6
0.8
1
1481
1623
1618
1619
1624
1625
peptide

Figure 13

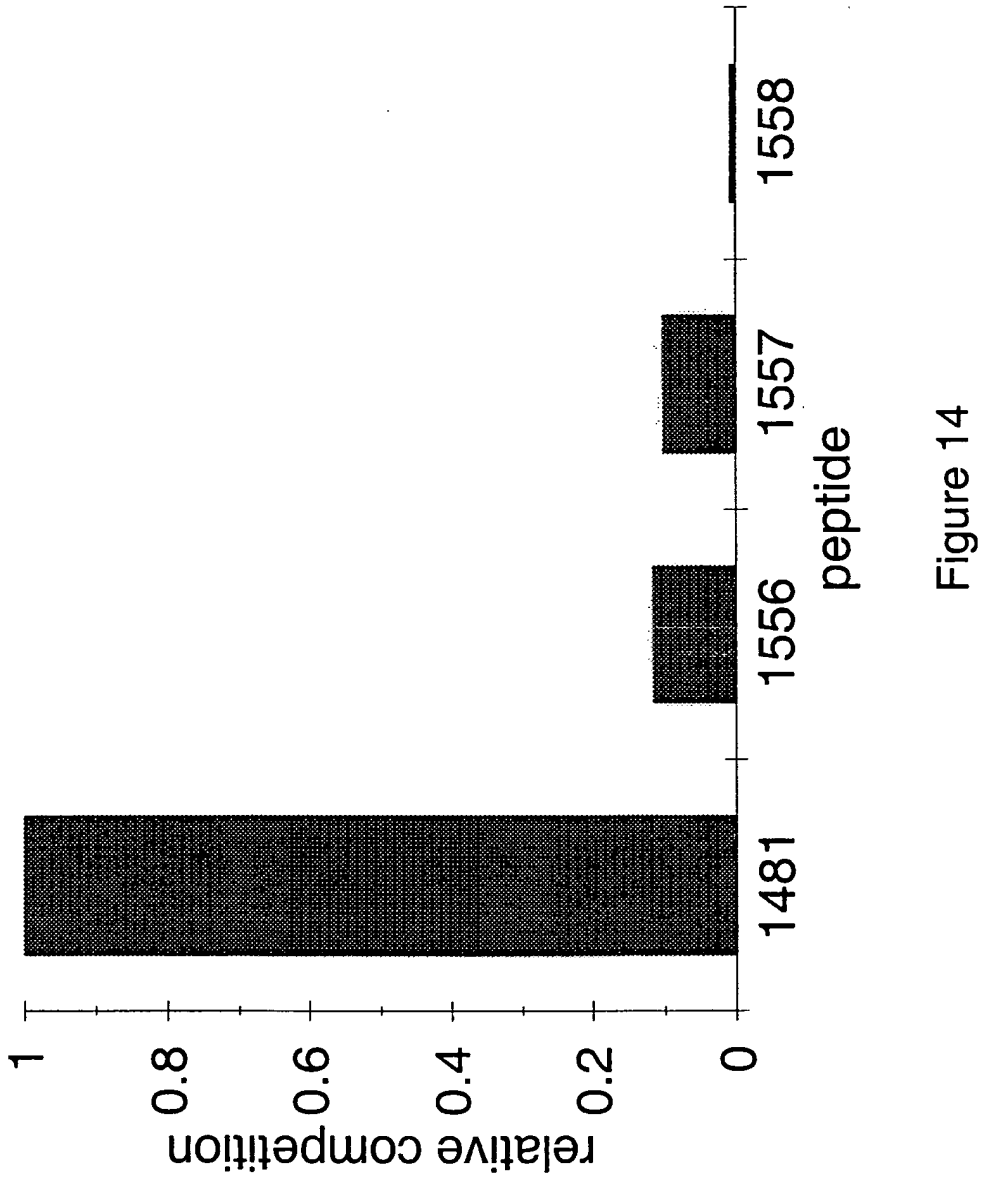
relative competition
0
0.2
0.4
0.6
0.8
1
1481
1556
1557
1558
peptide

Figure 14

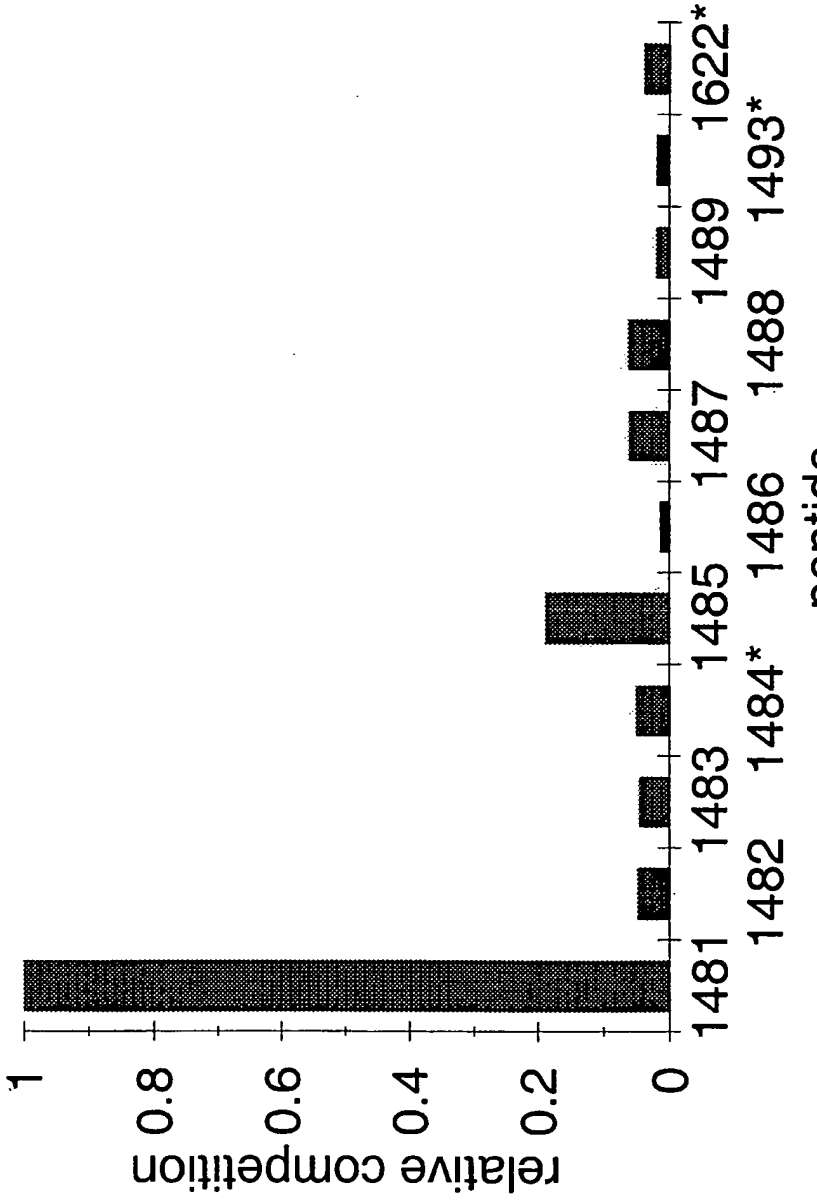
relative competition
0
0.2
0.4
0.6
0.8
1
1481
1482
1483
1484*
1485
1486
1487
1488
1489
1493*
1622*
peptide

Figure 15

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C07K 14/02, 14/045, 14/11, 5/078, 5/11, 5/103, A61K 38/07, 38/16, G01N 33/68, A61P 31/12

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(54) Title: BENZODIAZEPINES AND BENZOTHIAZEPINES DERIVATIVES AND HBSAG PEPTIDES BINDING TO ANNEXINS, THEIR COMPOSITIONS AND USE

(57) Abstract

The present invention relates to 1,4-benzodiazepines or 1,4-benzothiazepines derivatized with a peptide that can inhibit the interaction between annexin and annexin binding proteins. In particular, the present invention relates to 1.4-benzodiazepines or 1,4-benzothiazepines derivatives that can inhibit the interaction between annexin and viral proteins that bind annexins such as the HBaAg protein of HBV, glycoprotein B of the cytomegalovirus or any annexin binding protein from the influenza virus. These 1,4-benzodiazepines or 1,4-benzothiazepines derivates can be used to prevent or treat diseases in which interactions between annexin family members and annexin binding proteins are involved such as HBV and/or HBV infections, cytomegalovirus infections or influenza virus infections. The invention also relates to annexin binding epitopes from HBsAg and their use in the treatment of viral infections.

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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/EP 99/06231

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/02 C07K14/045 C07K14/11 C07K5/078 C07K5/11
C07K5/103 A61K38/07 A61K38/16 G01N33/68 A61P31/12

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOFMANN E.A.: "Interactions of benzodiazepine derivatives with annexins" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 5, 30 January 1998 (1998-01-30), pages 2885-2894, XP002098631 MD US cited in the application The whole document:see especially Fig.1:BDA452 ---	1-5,12, 16,17
X	WO 98 29442 A (YAP SING HIEN ;DEPLA ERIK (BE); INNOGENETICS NV (BE); MAERTENS GEE) 9 July 1998 (1998-07-09) The whole document; see especially page 11, lines 30 to page 13, line 11, example 13, table I --- -/--	9-13,16, 17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2000	30/03/00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/06231

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DE MEYER E.A.: "Characterization of small hepatitis b surface antigen epitopes involved in binding to human annexin v" J.VIRAL HEPATITIS, vol. 6, 1999, pages 277-285, XP000867365 the whole document	9-13,16, 17
X	HONORATI E.A.: "Epitope specificity of Th0/Th2 CD+ T-lymphocyte clones induced by vaccination with rHBsAg vaccine" GASTROENTEROLOGY, vol. 112, no. 6, June 1997 (1997-06), pages 2017-2027, XP000891211 The whole document; see especially fig.3 the whole document	9-13,16, 17
A	FR 2 479 818 A (ROUSSEL UCLAF) 9 October 1981 (1981-10-09) the whole document	
A	NACHMAN E.A.: "Synthesis, biological activity, and conformational studies of insect allatostatin neuropeptide analogues incorporating turn-promoting moieties" BIOORG.MED.CHEM., vol. 6, no. 8, 1998, pages 1379-1388, XP002098632 cited in the application the whole document	
A	WO 94 01554 A (INNOGENETICS NV ;YAP SING HIEM (BE)) 20 January 1994 (1994-01-20) cited in the application the whole document	
A	HERTOGS E.A.: "Endonexin II present on human liver plasma membranes, is a specific binding protein of small HBV envelope protein" VIROLOGY, vol. 197, 1993, pages 549-557, XP002098633 cited in the application the whole document	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/06231

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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2. ☐ Claims Nos.:
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3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

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☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8,14(all complete),10-13,16,17(all partially)

   Compounds as defined in claim 1, their compositions and use and the use defined in claim 14

2. Claims: 9,15(all complete),10-13,16,17(all partially)

   peptides as defined in claim 9, their compositions and use and the use defined in claim 15

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/EP 99/06231

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9829442 A	09-07-1998	AU 5860198 A	31-07-1998
		EP 0948533 A	13-10-1999
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